Northeastern Area State & Private Forestry

P-72-2 5230

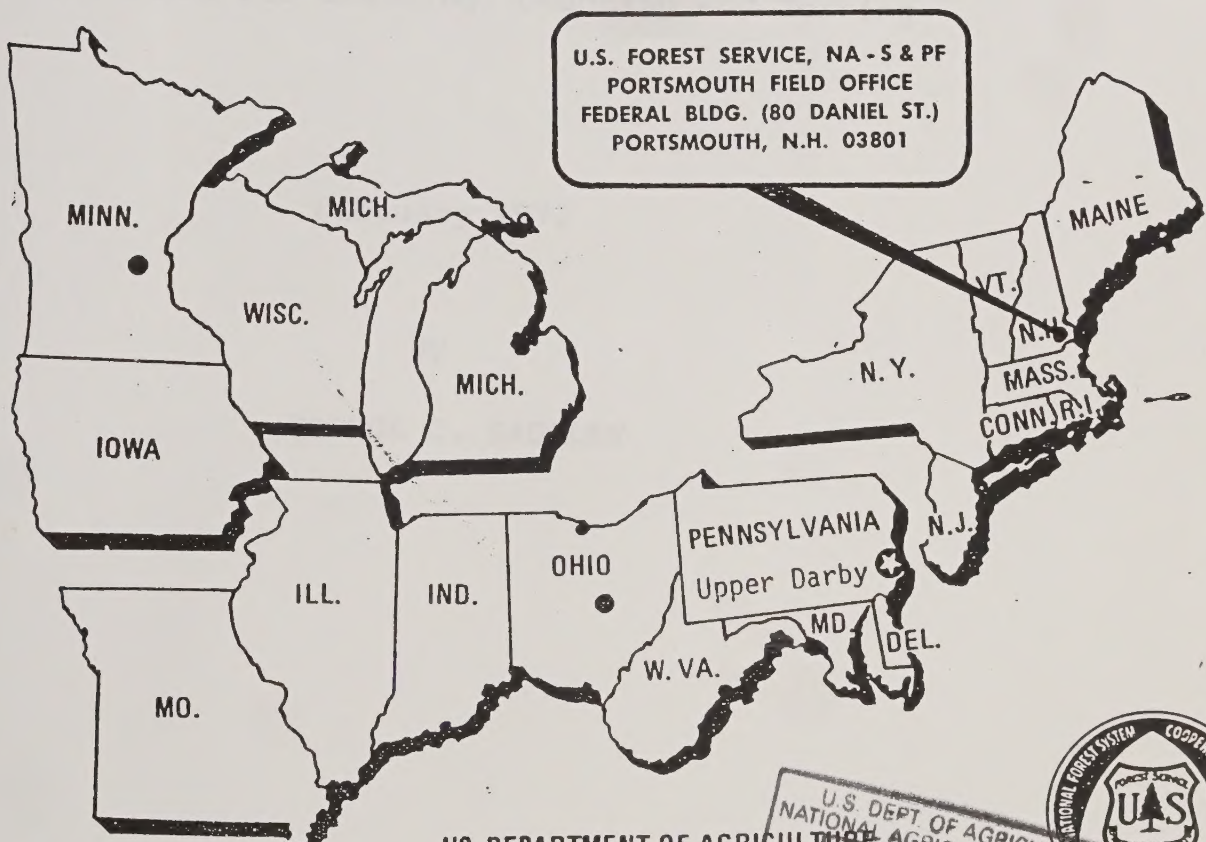
GYPSY MOTH EGGS

A METHOD FOR CLEANING, COUNTING AND SORTING

FEBRUARY 1972

BY

GEORGE C. SAUFLEY



US DEPARTMENT OF AGRICULTURE
FOREST SERVICE

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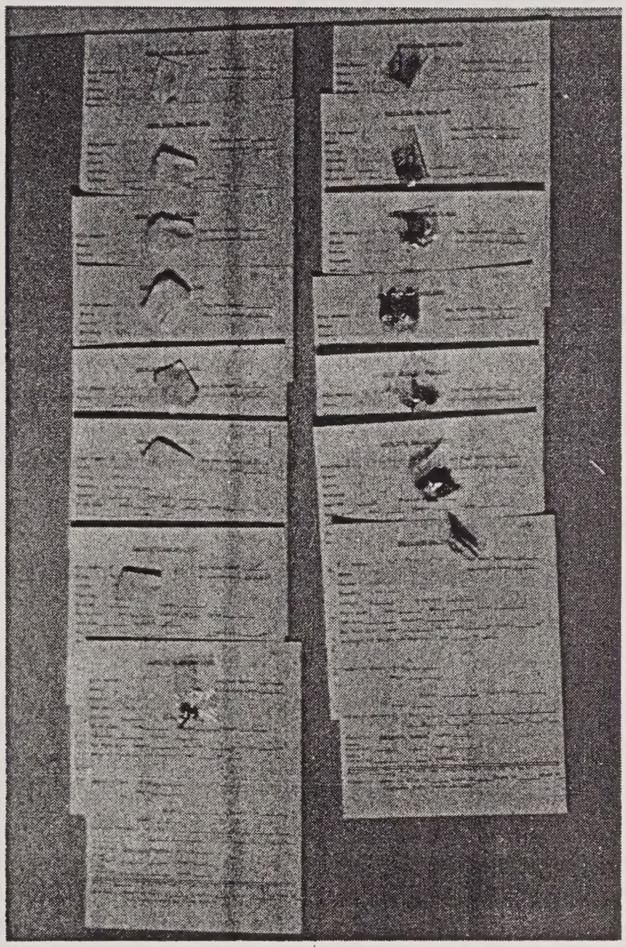


Photograph 1
Gypsy Moth Egg masses for processing
with corresponding data sheets.

INTRODUCTION

In 1971 a plan was developed to evaluate the effectiveness of the 1971 suppression projects against the gypsy moth in New Jersey, New York and Pennsylvania. A supplement to the work plan, October 1971, described the procedures for collecting and processing gypsy moth egg masses. This report describes the equipment and methods for "de-hairing", counting, and sorting the eggs referred to on pages 3 and 4 of the work plan supplement.

OBJECTIVES

1. To remove the major portion of "hair" that surrounds the eggs in a gypsy moth egg mass.
2. To count the number of eggs in individual egg masses by a rapid method.
3. To sort the eggs into three major categories:
    a. viable eggs
    b. parasitized eggs
    c. aborted eggs

METHODS AND MATERIALS

1. "De-hairing" and sorting apparatus and use of our de-hairing machinery was patterend after Pete Minor's device used in New Jersey. It consisted of a vacuum cleaner and a home-made attachment as shown in photograph 2. The vacuum cleaner was a Sears* model 11621891 canister type with flexible hose. (Figure 1A) The attachment consisted of two ordinary "tin" cans, made up in the following manner:
    a. A 1¼ hole was made through the center of the bottom of can "A". A 1¼" diameter brass tube 2" long was soldered against the hole in the can to make a funnel-like

* Use of a brand name does not imply endorsement of a product.

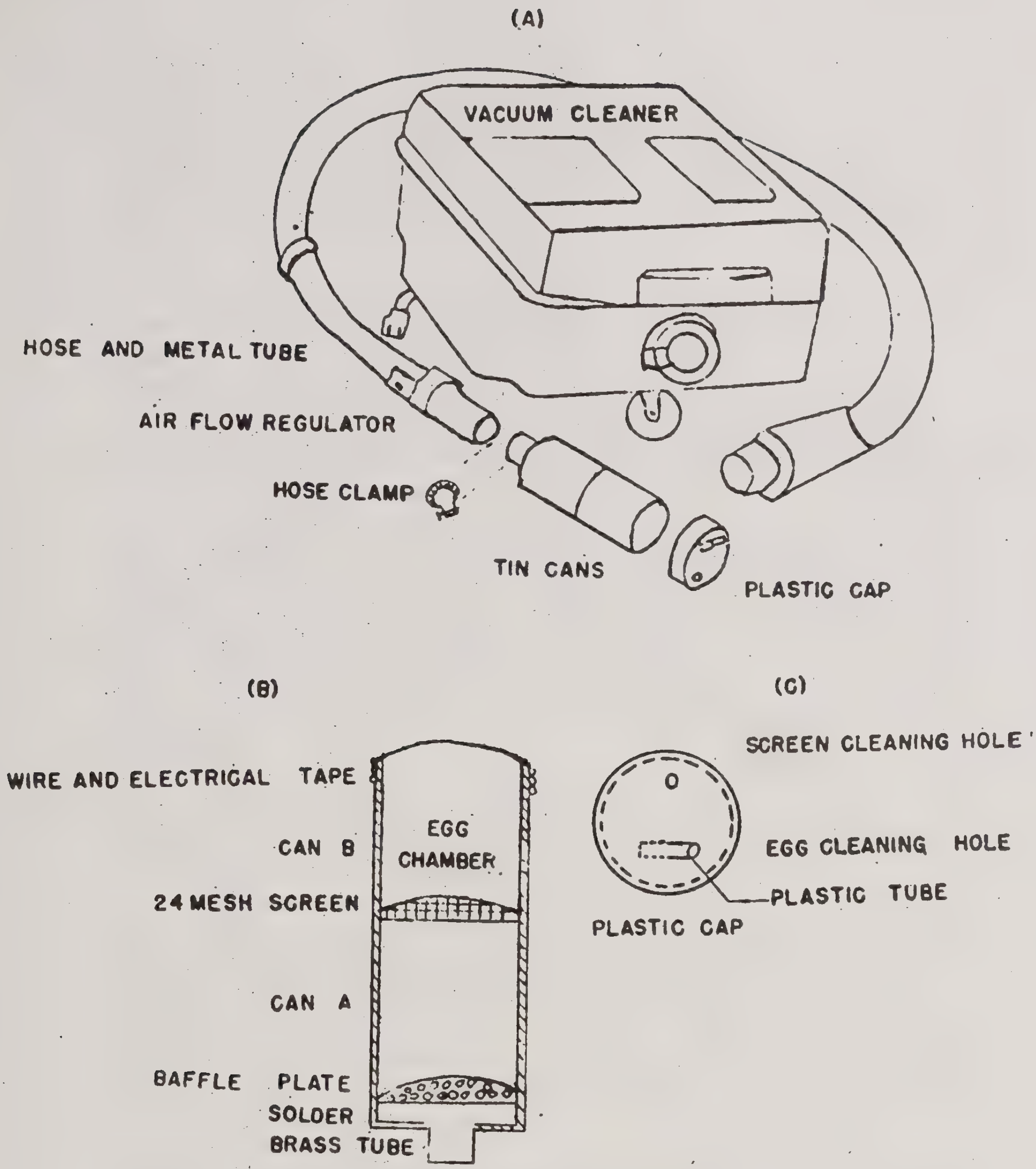


Figure 1. Apparatus for "de-hairing" gypsy moth egg masses. (A) Vacuum cleaner with tin can attachment. (B) Detail of tin can attachment. (C) Cap for egg chamber of tin can attachment.

arrangement. It was necessary to coat the entire bottom of the can with solder to insure stability. The outer coating was removed from places on the can where solder was applied.

b. A baffle plate was cut to the diameter of can "A" then fastened with silicone cement inside the can ½" from, and parallel to the bottom of the can. The plate contained 300 evenly distributed holes, each 1/16" in diameter. This baffle plate provided better distribution of air currents within the can.

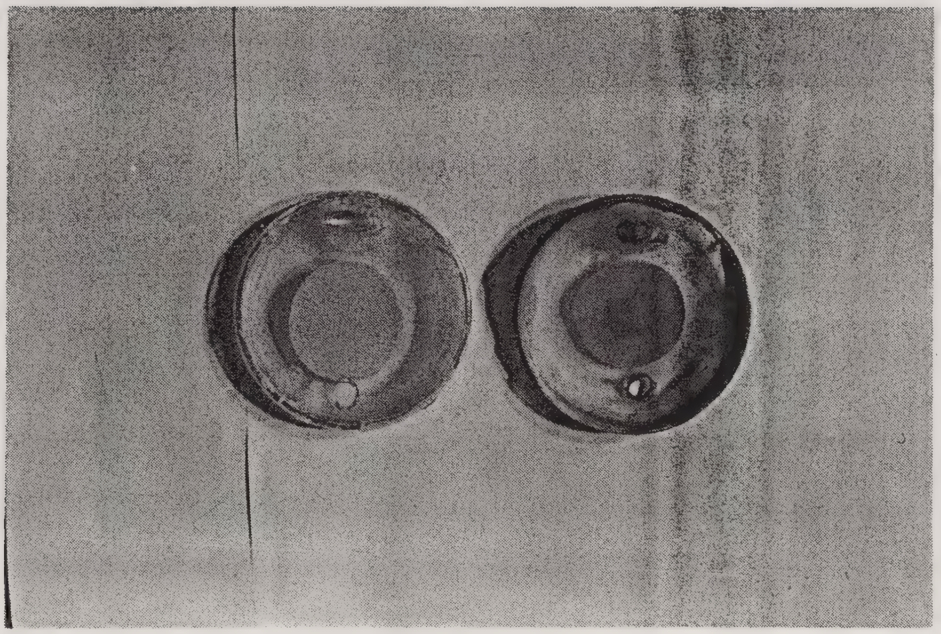
c. Can "B" had top and bottom removed and the bottom replaced with a 24 mesh screen affixed with silicone cement. The top lid of can "B" was protected against wear and tear by three turns of bare number 12 wire covered with four turns of electrical tape wrapped around the outside.

d. The bottom opening of Can "B" was soldered and taped to the top opening of can "A" to form a cylinder as shown in Figure 1B.

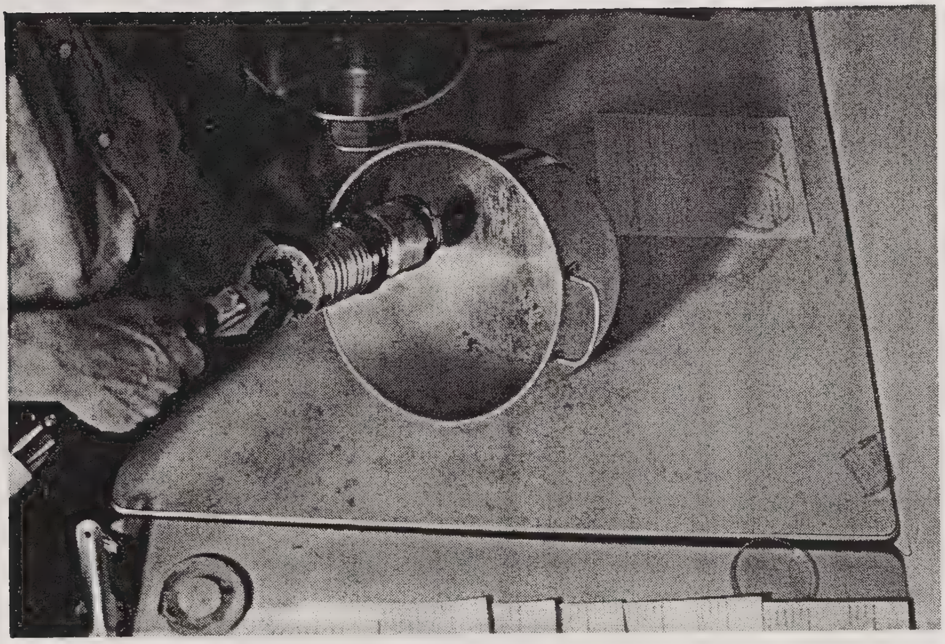
e. The brass tube on the bottom of can "A" was slipped over the attachment end of the vacuum cleaner hose and held in place with a hose clamp. The clamp was wrapped with electrical tape to make a smooth grip. It was necessary to cut and flare the brass tube for a good fit over the vacuum hose.

f. The bottom of a plastic petri dish was inverted and epoxy-glued to the inside of the petri dish top. This plastic lid was placed over the top of can "B" to prevent escape of gypsy moth eggs as they bounced around in can "B" during "de-hairing". Air was admitted through two 1/3" holes drilled through the plastic lid. A ½" piece of ¼" plastic tubing was glued into one hole at a 30° angle in order to direct air flows around the edge of the can. Each hole was put near the edge of the lid. (Figure 1C, Photograph 3)

Photograph 2
Dehairing and sorting machine made from vacuum cleaner.



Photograph 3
Top and bottom view of plastic cap used for cleaning and dehairing eggs.



Photograph 4
Sorting and dehairing gypsy moth eggs by tapping the tin can device on the side of an aluminum kettle.

The "de-hairing" apparatus was operated in the following manner:

a. The egg cluster was placed in Can "B", and the plastic lid was put on.

b. The vacuum cleaner was turned on with its adjustable suction control valve on the hose in the ½ closed position.

c. The plastic lid was rotated 3 to 4 times while the eggs bounced about in Can "B" and the hairs were sucked through the screen into the vacuum cleaner.

d. When the operator saw that the hair was cleared away, he opened the suction control valve, inverted Can "B" in a four-quart aluminum kettle, removed the plastic lid, then tapped Can "B" against the inside of the kettle to collect the viable eggs.

e. The operator next inverted Can "B" in another four-quart kettle, turned off the vacuum cleaner, then tapped Can "B" against the inside of the kettle to collect parasitised and aborted eggs. (Photograph 4)

   It required about 30 seconds to de-hair and sort the eggs into the two groups of viable and others plus bark flakes. The counting device described next was used to separate eggs from trash.

2. Egg Counting Equipment and Use

   A pipette graduated in hundreds of milliliters was used to count eggs volumetrically. A sample of 720 viable, de-haired eggs was put into the Kimble disposable pipette. The 720 egg count was then divided by the volume in milliliters to arrive at a mean number of eggs per 0.01 ml. Values for other volumes were calculated by cumulation of the mean, and arranged in a number to volume ratio chart. Appendix A shows egg counts for various volumes of viable eggs and Appendix B shows counts for

The "de-watering" [illegible] was [illegible] in the following manner:

3. The [illegible] placed [illegible], the plate [illegible]

4. The vacuum [illegible] adjustable [illegible] hose [illegible]

The [illegible] with the [illegible] the [illegible] into [illegible]

6. When [illegible] plate [illegible] cloth [illegible] control [illegible] with [illegible] at [illegible] the [illegible]

5. The [illegible] four-quart [illegible] placed [illegible] then [illegible] of the kettle [illegible] eggs. (Photograph 4)

It required about 30 seconds to [illegible] and sort the eggs into the two groups [illegible] and others plus dark flakes. The [illegible] described next was used to separate eggs from trash.

2. Egg Counting Equipment and Use

A pipette graduated in hundredths of milliliters was used to count eggs volumetrically. A sample of [illegible] viable, de-haired eggs was put into the [illegible] pipette. The [illegible] egg count was then divided by the [illegible] in milliliters to [illegible] number of eggs per 0.01 ml. [illegible] volumes [illegible] and [illegible] of the mean. [illegible] volumes

other eggs. The reliability of the charts was tested during the first week of use by random selection of 26 samples. Chart values and actual counts were subjected to a paired t test. There was no significant difference at the 95% level. Calculations are shown in Appendix C.

The egg counter consisted of a pipette, a siphon valve, a plastic tube, and a vacuum pump arranged as in Figure 2. The pump was a model 500 58 AF 713 fitted with a four foot length of ¼-inch poly propylene tubing. (Photograph 5) The siphon valve (S.P. TRU Flate) was attached to the free end of the tube for easy control of vacuum. The pipette was fitted at the base with a ½-inch length of plastic tubing to insure an air-tight joint with the siphon valve. A piece of 24 guage copper screening was coiled and placed in the pipette at the zero mark to prevent loss of eggs into the vacuum pump.

The counting of eggs immediately followed the de-hairing and sorting procedures. The pipette, with vacuum pump on, was moved about in the kettles described above in order to pick up all eggs while avoiding bark flakes. (Photograph 6)

When all eggs in a kettle were picked up by the pipette, the volume was read and the number of eggs determined from the appropriate chart. (Appendix A and B) (Photograph 7) The number was recorded on the data sheet. (Appendix D) Plastic 10 dram vials were used to receive the eggs from the pipette by putting the tip of the pipette into the vial, releasing the vacuum, and tapping the pipette against the vial.

3. Separation of Parasitised, Hatched and Aborted Eggs

Following the sorting of eggs into two major groups of viable and others, it was found that some viable eggs were being held by the vacuum

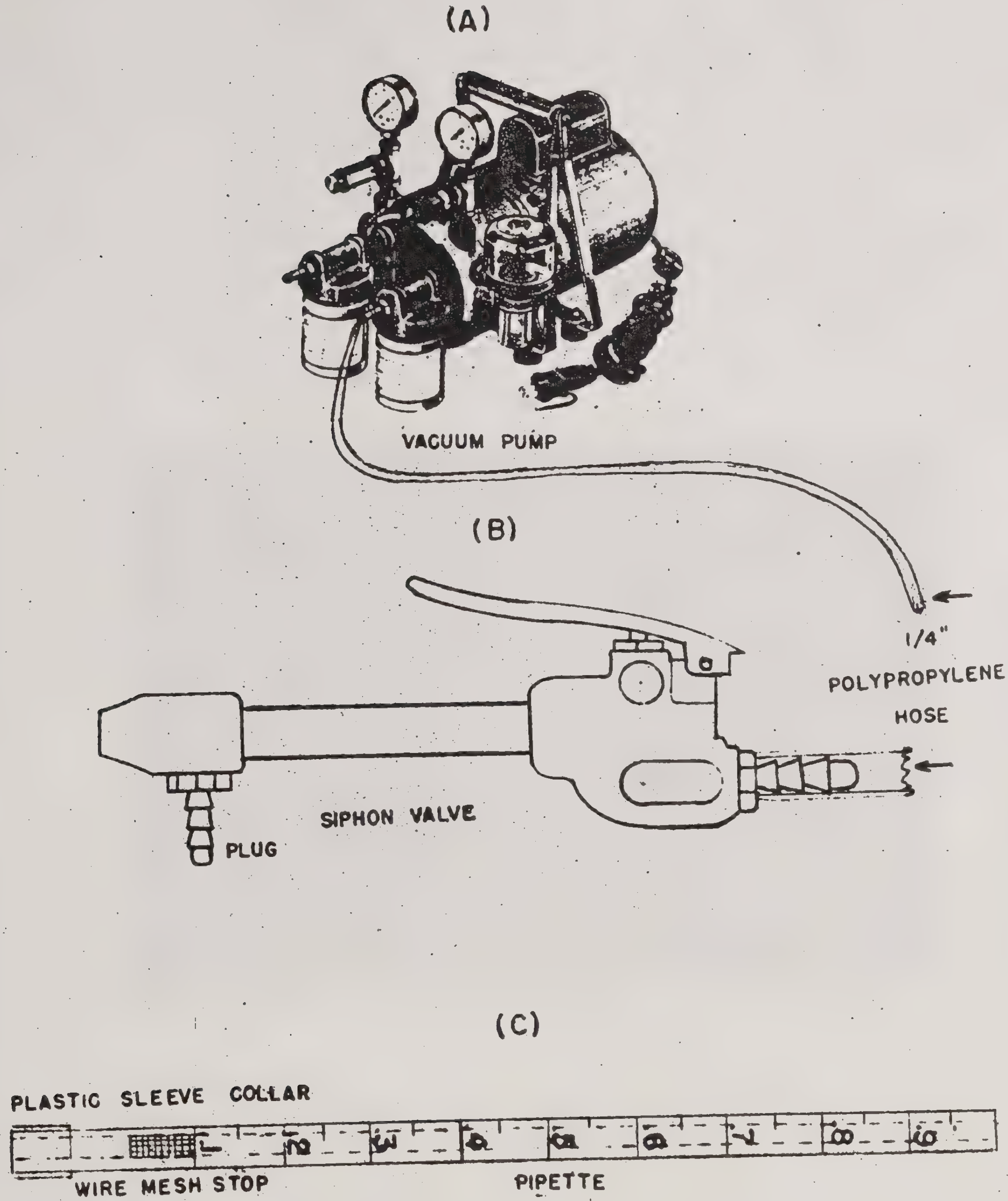
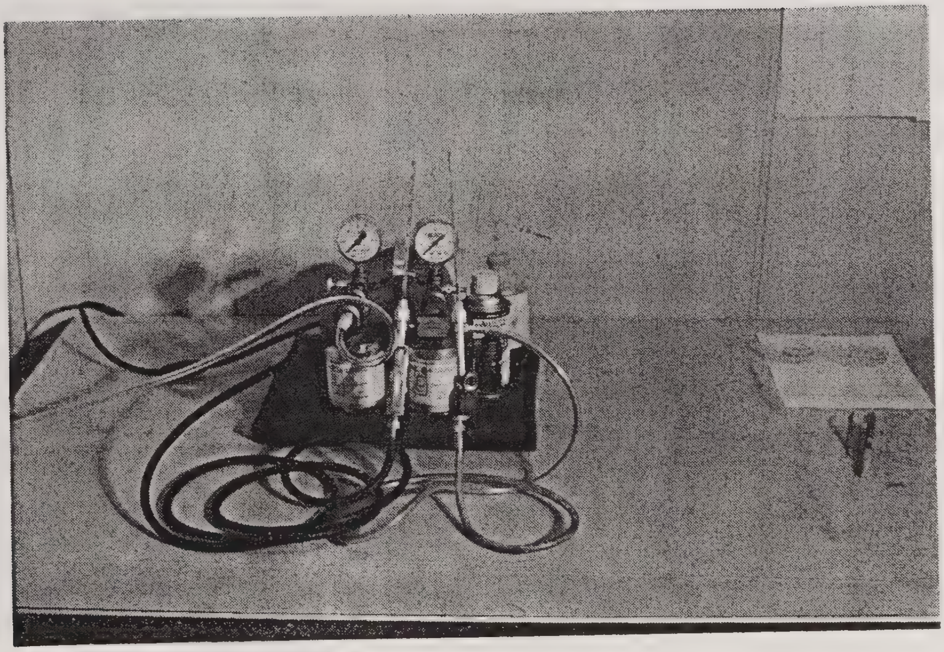
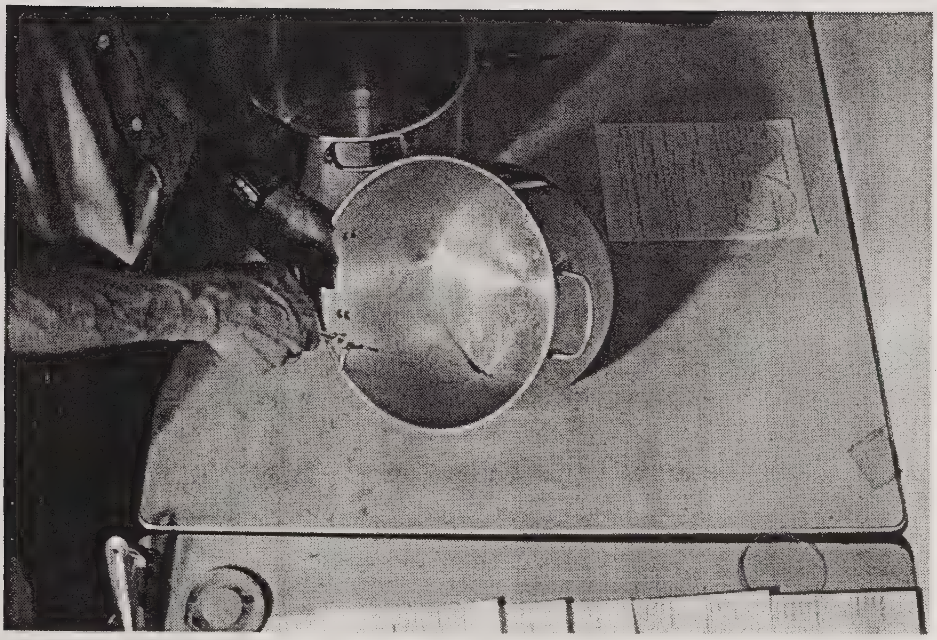


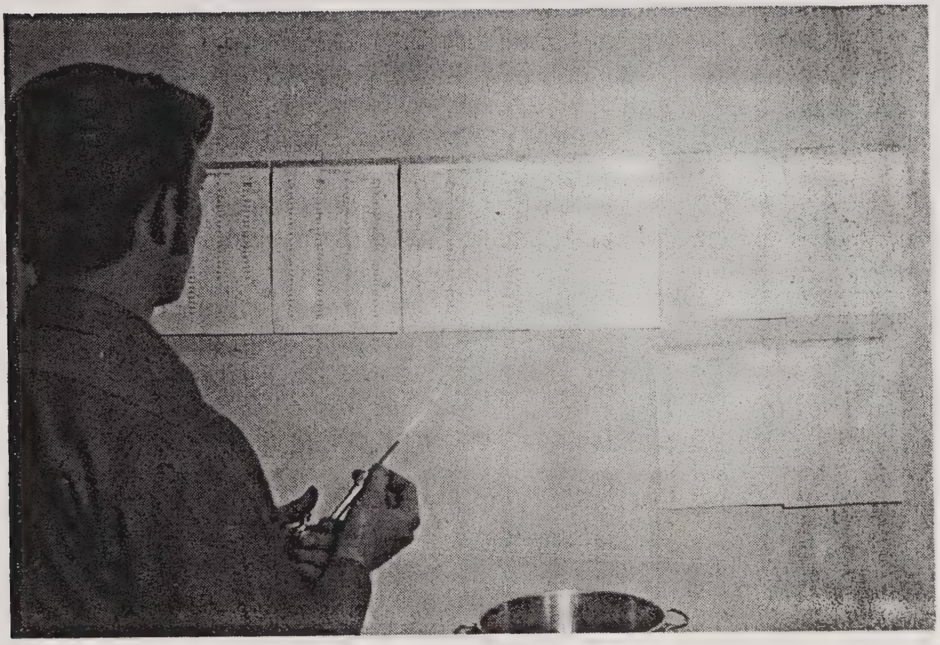
Figure 2. Gypsy moth egg counter (A) vacuum pump (B) siphon valve (C) pipette with plastic collar and wire mesh egg stop.



Photograph 5
Vacuum pump used to count gypsy moth eggs and sort out bark scraps.



Photograph 6
Forestry aid using pipette to pick up and count gypsy moth eggs while avoiding bark flakes.



Photograph 7
Forestry aid counting eggs using the pipette and number to volume ratio charts.

cleaner attachment so that they were placed in the vial with aborted and parasitised eggs. While this did not interfere with the job at hand, it could mean loss of some viable eggs in later work. A Chi Square Test was applied on 90 samples to test for a significant loss of viable eggs to the "other" classification. There was no significant difference at the 95% level as shown in Appendix E.

It was necessary to examine the "other" under a 150 power binocular microscope for separation into groups of hatched, parasitised and aborted eggs. Photographs 8, 9, 10, 11 and 12 shows typical examples of the three categories. A parasitised egg sometimes contained a cadaver of *Ooencyctus kuwanai*, but the cause of mortality and generation involved was not determined. In all but two cases, the *O. kuwanai* were adults. Rarely were two dead adult *O. kuwanai* found in a single gypsy moth egg. Of the aborted eggs, 80% contained mummified caterpillars, 10% were occupied by fungus mycelium, and 10% by undeveloped yolk. In one case, all eggs within the egg mass were undeveloped; and in another case, there were no eggs in the egg mass.

CONCLUSIONS

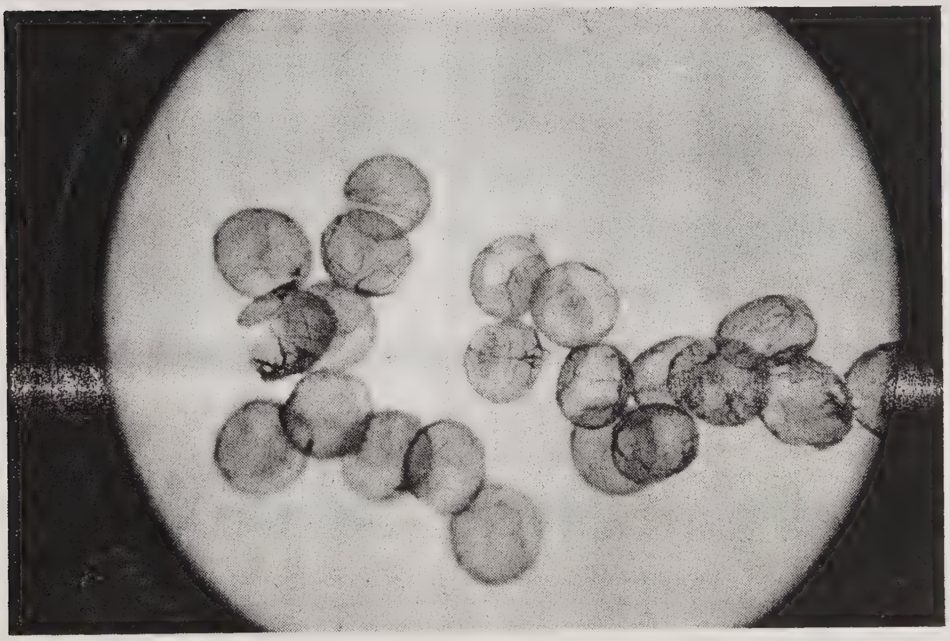
After using the described apparatus on 2,500 gypsy moth egg masses, we conclude that:

1. The use of a vacuum cleaner and home-made screen device provided a rapid, thorough, and accurate method for "de-hairing" and sorting of gypsy moth eggs into groups of viable and "other" eggs.

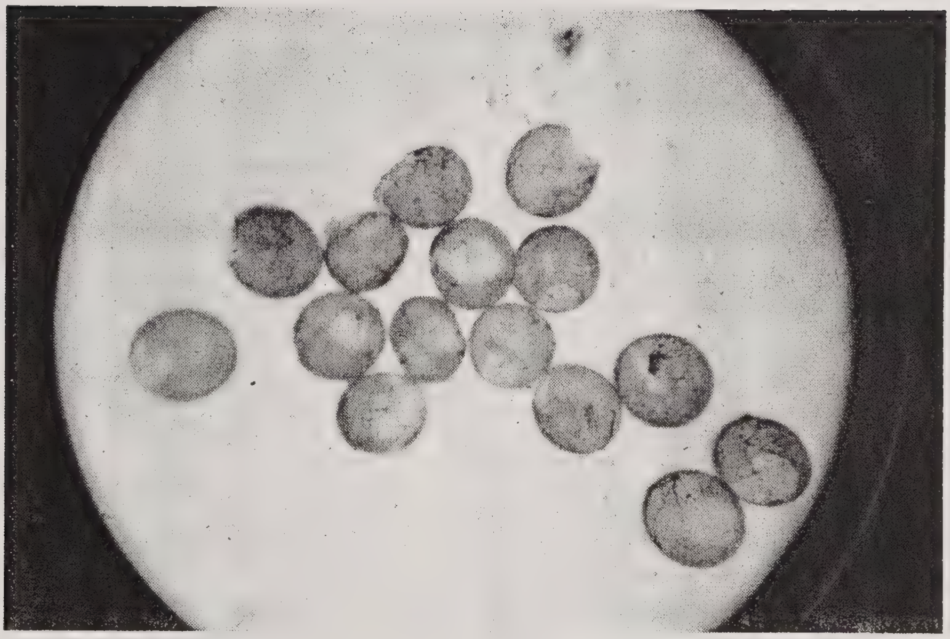
2. The vacuum pump-pipette apparatus provided a rapid, accurate method of counting gypsy moth eggs. It also simplified the handling of eggs as well as providing a way to further separate eggs from debris.

3. Some humidity is necessary in the processing room because static electricity may accumulate on the surface of the eggs causing problems that interfere with the transferring and counting of the eggs.

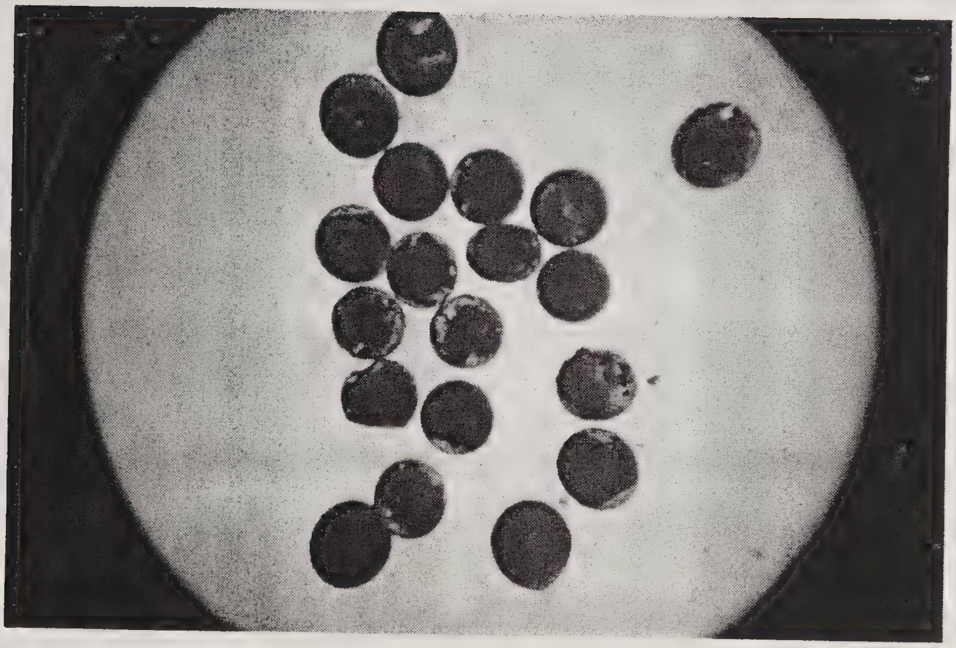
The ocular inspection method was slow and tedious; but it was found to be an accurate way of separating non-viable eggs on the basis of parasitism, hatch, and non-viability.



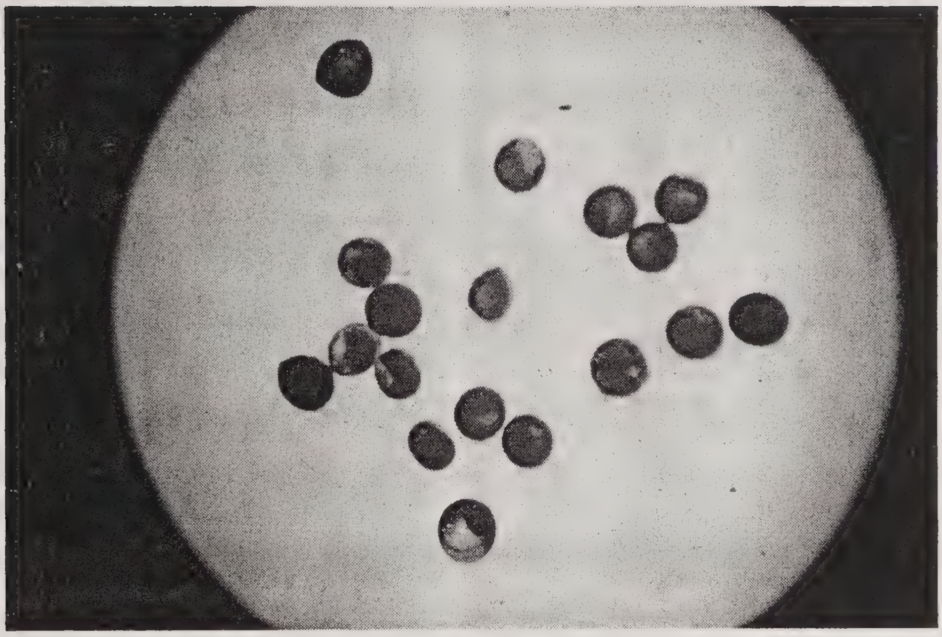
Photograph 8
Gypsy Moth Eggs After Hatch



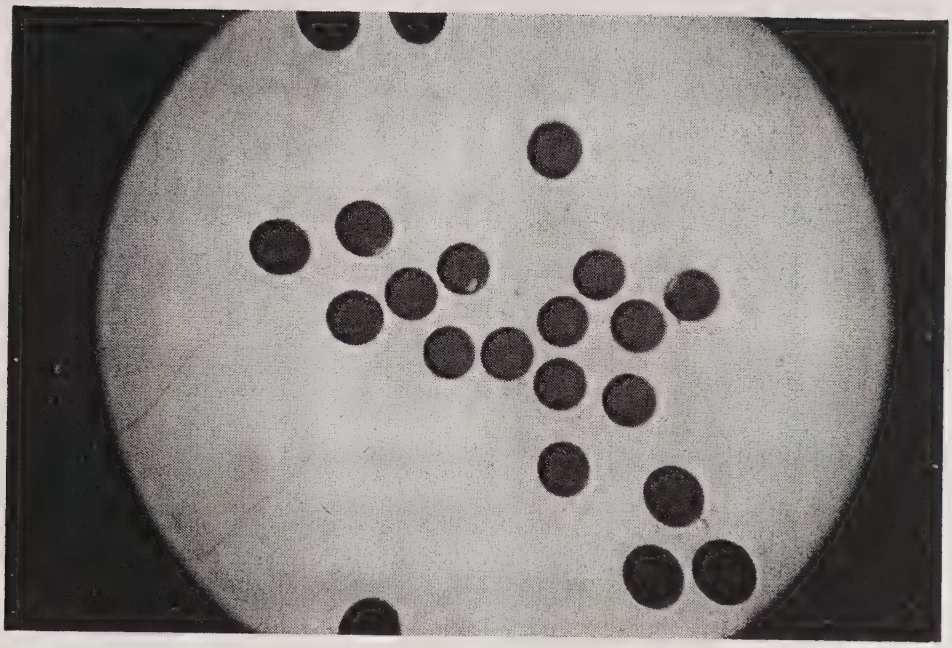
Photograph 9
Gypsy Moth Eggs Following Ooencyrtus kuwanai emergence



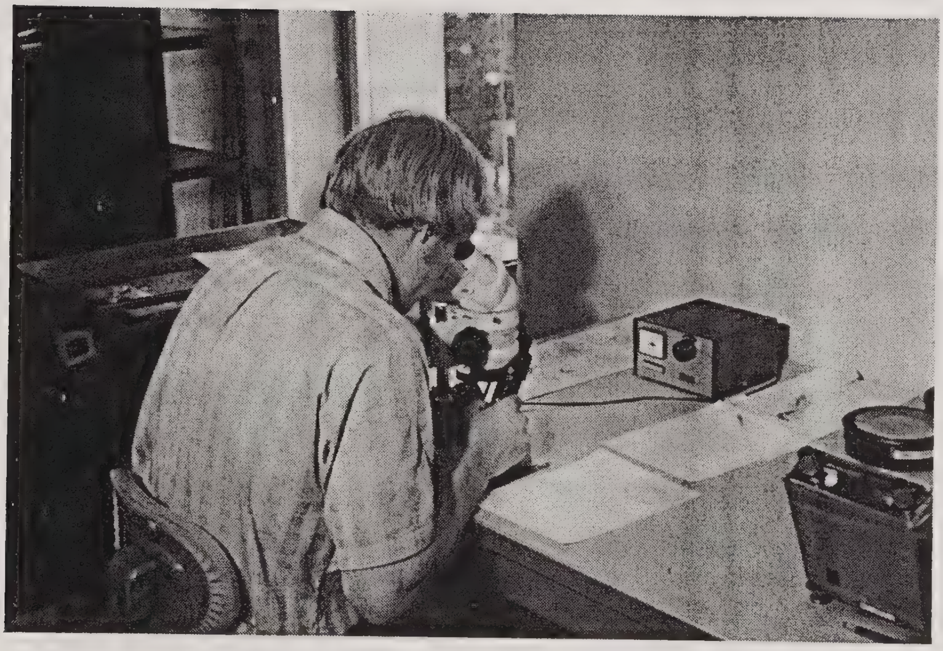
Photograph 10
Gypsy Moth Eggs Containing Dead *Ooencyrtus kuwanai*



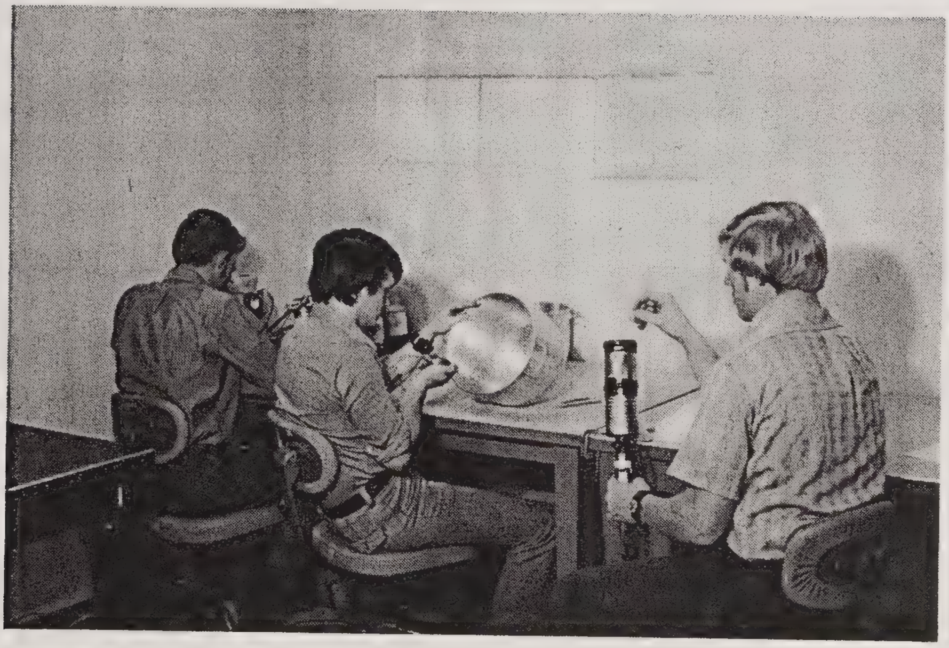
Photograph 11
Aborted Gypsy Moth Eggs



Photograph 12
Viable Gypsy Moth Eggs



Photograph 13
Forestry aid separating gypsy moth eggs with the aid of a binocular microscope.



Photograph 14

Left to right, Forestry Aids, Gary Hare, John Magoon, and Bruce Anderson processing gypsy moth eggs using apparatus described in this report.

APPENDIX A

Gypsy Moth Eggs
Number to Volume Ratio
in 1/10 ML. Tubes

VIABLE EGGS

ML	No. Eggs in Tube	ML	No. Eggs in Tube
.01	7.7	.27	207.9
.02	15.4	.28	215.6
.03	23.1	.29	223.3
.04	30.8	.30	231.0
.05	38.5	.31	238.7
.06	46.2	.32	246.4
.07	53.9	.33	254.1
.08	61.6	.34	261.8
.09	69.3	.35	269.5
.10	77.0	.36	277.2
.11	84.7	.37	284.9
.12	92.4	.38	292.6
.13	100.1	.39	300.3
.14	107.8	.40	308.0
.15	115.5	.41	315.7
.16	123.2	.42	323.4
.17	130.9	.43	331.1
.18	138.6	.44	338.8
.19	146.3	.45	346.5
.20	154.0	.46	354.2
.21	161.7	.47	361.9
.22	169.4	.48	369.6
.23	177.1	.49	377.3
.24	184.8	.50	385.0
.25	192.5	.51	392.7
.26	200.2	.52	400.4

APPENDIX A - CONTINUED

ML	No. Eggs in Tube
.53	408.1
.54	415.8
.55	423.5
.56	431.2
.57	438.9
.58	446.6
.59	454.3
.60	462.0
.61	469.7
.62	477.4
.63	485.1
.64	492.8
.65	500.5
.66	508.2
.67	515.9
.68	523.6
.69	531.3
.70	539.0
.71	546.7
.72	554.4
.73	562.1
.74	569.8
.75	577.5
.76	585.2
.77	592.9

ML	No. Eggs in Tube
.78	600.6
.79	608.3
.80	616.0
.81	623.7
.82	631.4
.83	639.1
.84	645.8
.85	654.5
.86	662.2
.87	669.9
.88	677.6
.89	685.3
.90	693.0
.91	700.7
.92	708.3
.93	716.1
.94	723.8
.95	731.5

APPENDIX B

Gypsy Moth Eggs
Number to Volume Ratio
in 1/10 ML. Tubes

HOLLOW EGGS

ML	No. Eggs in Tube	ML	No. Eggs in Tube
.01	7.57	.26	196.82
.02	15.14	.27	204.39
.03	22.71	.28	211.96
.04	30.28	.29	219.53
.05	37.50	.30	227.10
.06	45.42	.31	234.67
.07	52.99	.32	242.24
.08	60.56	.33	249.81
.09	68.13	.34	257.38
.10	75.70	.35	264.95
.11	83.27	.36	272.52
.12	90.84	.37	280.09
.13	98.41	.38	287.66
.14	105.98	.39	295.23
.15	113.55	.40	302.80
.16	121.12	.41	310.37
.17	128.69	.42	317.94
.18	136.26	.43	325.51
.19	143.83	.44	333.08
.20	151.40	.45	340.65
.21	158.97	.46	348.22
.22	166.54	.47	355.79
.23	174.11	.48	363.36
.24	181.68	.49	370.93
.25	189.25	.50	378.50

APPENDIX B - CONTINUED

ML	No. Eggs in Tube
.51	386.07
.52	393.64
.53	401.21
.54	408.78
.55	416.35
.56	423.92
.57	431.49
.58	439.06
.59	446.63
.60	454.20
.61	461.77
.62	469.34
.63	476.91
.64	484.48
.65	492.05
.66	499.62
.67	507.19
.68	514.76
.69	522.33
.70	529.90
.71	537.47
.72	545.04
.73	552.61
.74	560.18
.75	567.75

ML	No. Eggs in Tube
.76	575.32
.77	582.89
.78	590.46
.79	598.03
.80	605.60
.81	613.17
.82	620.74
.83	628.31
.84	635.88
.85	634.45
.86	651.02
.87	658.59
.88	666.16
.89	673.73
.90	681.30
.91	688.87
.92	696.44
.93	704.01
.94	711.58
.95	719.15

APPENDIX C

Paired t test for significant difference between hand counted and volumetrically counted gypsy moth eggs

Tube Sample A_1	Hand Samples B_1	$d.=A_1-B_1$	d^2
358	368	+10	100
100	109	+ 9	81
85	85	+ 0	0
43	43	+ 0	0
366	359	- 7	49
85	82	- 3	9
66	65	- 1	1
278	255	-23	1058
31	27	- 4	8
134	141	+ 7	49
207	195	-12	144
77	63	-14	196
546	466	-80	6400
108	105	- 3	9
139	157	+18	324
115	112	- 3	9
505	516	+11	121
136	150	+14	196
92	84	- 8	64
116	110	- 6	36
82	81	- 1	1
38	46	+ 8	64
172	177	+ 5	25

APPENDIX C - CONTINUED

124	120	- 4	16
255	218	+37	1369
30	30	+ 0	0
	SUM	-50	9807

$\bar{x}$ = 64.9 and 60.2 Calculated Value of *t* =1.191

Table value of *t* 95% =2.064

APPENDIX D

GYPSY MOTH EGG MASS DATA

Plot Number: Egg Mass Number:___

State : Collection Date:___

County :

Township :

Sprayed :____ Unsprayed: ____

Block size: Small____ Medium ____ Large ____

Isolated_________ Adjacent_______

Egg Mass size: Length___mm. width___ mm. Produce___ mm^2___

Selected for Weight and Diameter: Yes___ No.___

Number of Eggs: Total__________

Whole__________

Viable:________ Parasitized________

Blank:_________

Parasite Emergence: In Days 1___2___3___4___5___6___7___

8___9___10___11___12___13___14___

Parasites: <u>Ooencyrtus</u> <u>Kuwanai</u> Number_________

Other
Parasites_______ ________ Number________

Other
Parasites_______ ________ Number________

Weight:_______ m.g. (W.O. hair) includes all eggs in the mass)

Diameter: Number in Sieve 10____Sieve12___ Sieve 18___Sieve 20___
(includes viable and blank eggs)

APPENDIX E

CHI ^{2} TEST FOR SIGNIFICANT DIFFERENCE BETWEEN VACUUM SORTED AND HAND SORTED GYPSY MOTH EGGS

MACHINE SORTED	HAND SORTED	$d_2 - d_1$	Chi^2
277	291	-14	.673
177	177	0	0
184	203	-19	1.778
77	83	- 6	.433
123	130	- 7	.376
535	543	- 8	.117
131	135	- 4	.118
123	123	0	0
92	93	- 1	.010
89	96	- 7	.526
54	58	0	0
92	92	0	0
184	189	- 5	.270
855	857	- 2	.004
92	92	0	0
266	266	0	0
261	264	- 3	.034
108	109	0	0
115	115	- 1	.008
39	41	- 2	.097
85	85	0	0
324	324	0	0
149	155	- 6	.232

APPENDIX E CONTINUED

MACHINE SORTED	HAND SORTED	$d_2 - d_1$	Chi^2
254	267	-13	.631
64	65	- 1	.015
524	526	- 2	.001
489	492	- 3	.018
181	187	- 6	.192
758	760	- 2	.005
115	116	- 1	.008
768	770	- 2	.005
131	131	0	0
111	116	- 5	.215
41	49	- 8	1.300
69	69	0	0
724	724	0	0
69	74	- 5	.337
501	505	- 4	.031
334	335	- 1	.002
231	230	1	.004
589	588	- 1	.007
285	285	0	0
386	386	0	0
131	132	- 1	.007
103	105	- 2	.038
465	465	- 0	0
310	310	0	0
34	34	0	0
17	19	- 2	.210
262	261	+ 1	.003
116	117	- 1	.008

APPENDIX E CONTINUED

MACHINE SORTED	HAND SORTED	$d_2 - d_1$	Chi^2
62	61	+ 1	.016
146	145	+ 1	.006
108	106	+ 2	.037
123	125	- 2	.032
38	40	- 2	.100
592	615	-23	.860
261	264	- 3	.035
69	69	0	0
146	154	- 8	.415
77	83	- 6	.433
131	131	0	0
184	185	- 1	.005
262	262	0	0
462	462	0	0
932	932	0	0
77	75	- 2	.053
239	239	0	0
424	422	+ 2	.009
30	30	0	0
254	255	- 1	.003
100	102	- 2	.039
100	99	- 1	.010
130	129	+ 1	.007
130	129	+ 1	0
185	187	- 2	.021
0	0	0	0
46	46	0	0
177	177	0	0
124	124	0	0

APPENDIX E CONTINUED

MACHINE SORTED	HAND SORTED	$d_2 - d_1$	Chi^2
161	161	0	0
769	769	0	0
515	515	0	0
416	416	0	0
238	238	0	0
484	488	- 4	.032
385	385	0	0
516	519	- 3	.017
123	123	0	0

$x^2 = 9.856$

Table x^2 .05 = 113.14

GYPSY MOTH EGG MASS HATCH POTENTIAL METHODOLOGY

BY

GEORGE SAUFLEY

INTRODUCTION

Monitoring gypsy moth levels is typically done through pheromone trapping, burlap banding and/or egg mass counts. These techniques give the land managers a means to predict future populations and damage. They also can be used to help monitor general gypsy moth trends over a region or to estimate the occurrence of significant damage within individual stands.

By examining individual egg masses, predictions of population size and damage can be refined. However, this paper discusses ways of characterizing individual eggs of a gypsy moth egg mass. The methodology has been modified to be used along with estimates of egg masses per acre to assess potential egg hatch and parasitism in areas where high populations or changes in populations are expected.

Construction and operation of the dehairing-sorting apparatus was previously published in 1972. A description on the upgrades and changes in the equipment is available.

OBJECTIVES

1. To separate and sort eggs so that the health of the egg mass can be examined and egg mass condition evaluated as to its potential to hatch.

2. To distinguish eggs that will hatch from parasitized sterile or diseased eggs.

3. To use these estimates to assess egg mass health within geographic boundaries.

4. To incorporate these estimates into biological evaluations and control strategies.

MATERIALS AND METHODS

A binocular and compound microscope with a source of back lighting is needed to inspect the egg masses. An environmental chamber may be needed for quality control over the egg inspection process. Internal egg inspection for abnormalities, using back lighting, can be used to further categories egg viability.

To determine how many eggs can potentially hatch, examine the whole egg category. These whole eggs are examined under a binocular dissecting microscope usually at 6-12 power with an 8-10 X eye piece to get a wider field and examine more eggs. A 15 watt light bulb focused under the eggs will show a shadow of a caterpillar embryo. A group of 30-50 eggs is examined under a low power scope to get a ratio of eggs. An egg mass consists of caterpillar

embryos and eggs that have a potential for hatching (such as larval parasites, unfertilized and diseased eggs). This ratio is used to determine the potential hatch of the egg mass. Use as many fields as necessary to get a reasonable estimate of the condition of the egg mass.

A shadow of an embryo in the egg makes a statement about the egg's health. However, it is difficult to see into an egg without dissecting it. One solution is to place known healthy eggs one layer thick on a slide and with a dropper place diluted black ink between the eggs. This technique allows the examiner to see the eggs, cluster, contents and eventually get used to looking at egg clusters without ink. (Figure 1) If the egg mass cannot be easily separated into (2) distinct categories of eggs, then suspect the egg cluster's health. Both whole and hollow eggs may need examining to determine the cause. These egg masses are usually not healthy and have high levels of parasitism, winter kill or disease, but expect a small percentage of the eggs to hatch. If the population has been high, parasitism is increasing, the egg masses are small and cannot be separated into distinct categories by the dehairing equipment. Then a population collapse is likely because of parasitism and a depleted food supply. If the division between the whole and hollow is pronounced and the hollow eggs represent 1-10% of egg masses that are large and uniformly scattered throughout a preferred host stand; and if the whole category within the egg cluster indicates that 80% or more the egg cluster has the potential to hatch, then the population is likely to build. This condition may be supported by increased pheromone trap catch in the area.

Processed and unprocessed egg masses were reared in an environmental chamber to get an estimate of over wintering mortality and the results varied from

[illegible]

[illegible]). This ratio is used to determine the [illegible]

[illegible]

[illegible]

[illegible] a statement about the [illegible] health,

[illegible]

[illegible]

[illegible]

[illegible] eggs, [illegible]

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(2) distinct categories of [illegible]

[illegible] and hollow eggs [illegible] been sufficient to [illegible]

[illegible] usually not healthy [illegible] have high levels of parasitism

disease, but expect a small percentage of the eggs to [illegible]

[illegible] been high, [illegible]

[illegible] the population into distinct categories by the [illegible]

population collapse is likely [illegible]

[illegible]. If the distinction between the whole and hollow [illegible]

[illegible] the hollow eggs represent 1-10% of egg masses that are larger [illegible]

[illegible] throughout a preferred nest stand; and if the whole

[illegible] lesser indicates than 20% [illegible]

[illegible] batch, then the population is likely to build. This [illegible]

[illegible] by increased [illegible] crop eaten in the area.

[illegible]

[illegible] egg masses [illegible]

[illegible]

location to location and with IN populations. Comparisons of processed and unprocessed egg mass classes according to size were also made to see if the occular method was a reliable indication of egg health. Eggs used in the comparison were hatched in an environmental chamber. When observations were averaged, there was no difference between those that were expected to hatch and those eggs that actually hatched.

When the sorting and inspection was complete, the remainder of whole eggs were placed in petri dishes, labeled, and placed in a styrofoam box along with damp paper toweling sprayed with 1% sodium benzoate solution and stored in a walk-in cooler for 3 months around 34°. Next the eggs were removed and reared in an environmental chamber at 80° F 70% RH at 14, 10 photo period. Gypsy moth eggs have been reared under a number of parameters. If the average temperature and RH approximately early summer weather the eggs will hatch. The remaining eggs from the inspection process are a better indicator of hatch because prolonged exposure to intense light will damage the eggs.

Both whole and hollow eggs may be examined under a 150 power compound binocular microscope for hatch potential. Hatched and emerged parasitized eggs as well as completely dry eggs are separated out with the hollow eggs. Parasites that have not emerged or eggs containing most of their original weight that are diseased may drop as whole eggs. These are examined when the whole eggs are scoped. The dark brown known caterpillars in the eggs are gypsy moth larvae. If a lot of white or light brown larvae show up in the egg cluster, they should be reared for parasites. (Figure 2)

Potential egg mass hatch is determined by the following steps:

a) Dehair, sort and volumetrically count whole and hollow eggs. Whole + hollow may be scoped if necessary, to determine the ratio of healthy eggs per mass.

b) Total eggs per mass = whole + hollow eggs.

   1) From whole eggs examine 50 or so eggs that comfortably fit into microscope field. Examine 1 or 2 fields.

c) # est viable eggs in scope fields = total eggs in scope fields minus eggs examined that will not hatch. (Formula can be other way around.)

d) Ratio of eggs that will hatch = <u>est viable eggs in scope fields</u>/total eggs examined in scope fields.

e) # est eggs hatch per mass = total whole eggs times percent of eggs that will hatch.

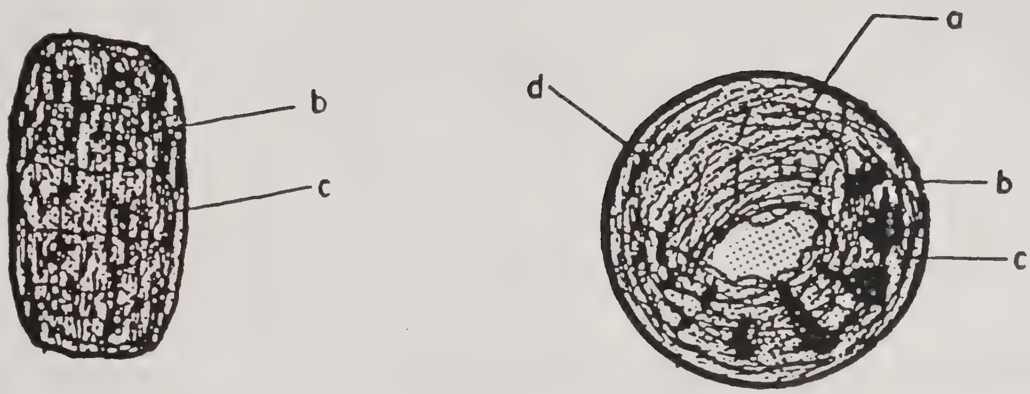
f) % of eggs that will hatch per mass = <u># est eggs hatch per mass</u>/total eggs per mass x 100.

g) Percentages on egg mass hatch and observations on egg health are used to recalculate estimates from egg mass cruses in the field.

If a computer or programmable calculator is used, only the measurements and scope estimates are entered into the machine and the computer prints out the results while another egg mass is being processed or scoped. The computer and printer need not be expensive because the program is short.

RESULTS

Dehairing, sorting and estimating the proportions of eggs in a gypsy moth mass that have the potential to hatch in a geographical area is an indication of the health of egg masses for that area. The number of percent of eggs that will hatch in an egg mass can be incorporated into on-going biological evaluations to improve the chances of predicting the outcome of a gypsy moth population. A more accurate prediction scheme gives the land manager owners a wider choice of alternatives when controlling the insect or protecting his resources (and a more reliable prediction system) provides a greater chance for a better benefits cost ratio with gypsy moth control.



a) Shadow head capsule

b) Internal organs, structures inside egg

c) Thorax and abdomen

d) Larval hairs

Figure 1--Examples of gypsy moth embryos in eggs.

a | b | c

Parasite Embryos
Lighter in color/white

O. Kuanai Emergence Hole

Gypsy Moth
Emergence Hole

(In most cases parasite is difficult to see in egg because of hairs, egg structures)

a^1 *Ocancyrtus kawanai*

a^2 *Anastatus bifasciatus*

Figure 2--a) Containing parasites

b) *O. Kuanai* Emergence Hole

c) Gypsy Moth Emergence Hole

INSTRUCTIONS FOR BUILDING
GYPSY MOTH DEHAIRING-SORTING APPARATUS

ASSEMBLING A DEHAIRING-SORTING APPARATUS

1. The dehairing-sorting apparatus consisted of a vacuum cleaner, dehairing chambers, gate valve, swivel joint, stand and collecting pot. The dehairing head consists of copper pipe, made up in the following manner:

   A 6 3/4 inch copper pipe, 3 inches in diameter, is cut into 2 pieces, 3 3/4 and 3 inches long. At one end of the shortest piece a 3 inch to 1 1/2 inch copper or brass reducer is attached. Inside the pipe, one inch from the reducer, a baffle plate with 1/8 inch evenly distributed holes is attached. At the other end of the 3 inch pipe, a 24-30 mesh screen is attached and then fastened to the 3 3/4 inch pipe. All joints can be soldered with plumber's solder. Half way up the 3 inch pipe section a 1/2 inch hole is drilled to form a clean out port. The hole is taped shut when processing eggs. Four 1/4 inch evenly distributed square notches are cut in the open end of the 3 3/4 inch pipe. A 1 1/2 inch x 1 1/2 inch nipple is soldered to the reducer for attaching to vacuum cleaner and rack. A petri dish is placed on the notched end of the dehairing chamber when the equipment is used. (Figure 1)

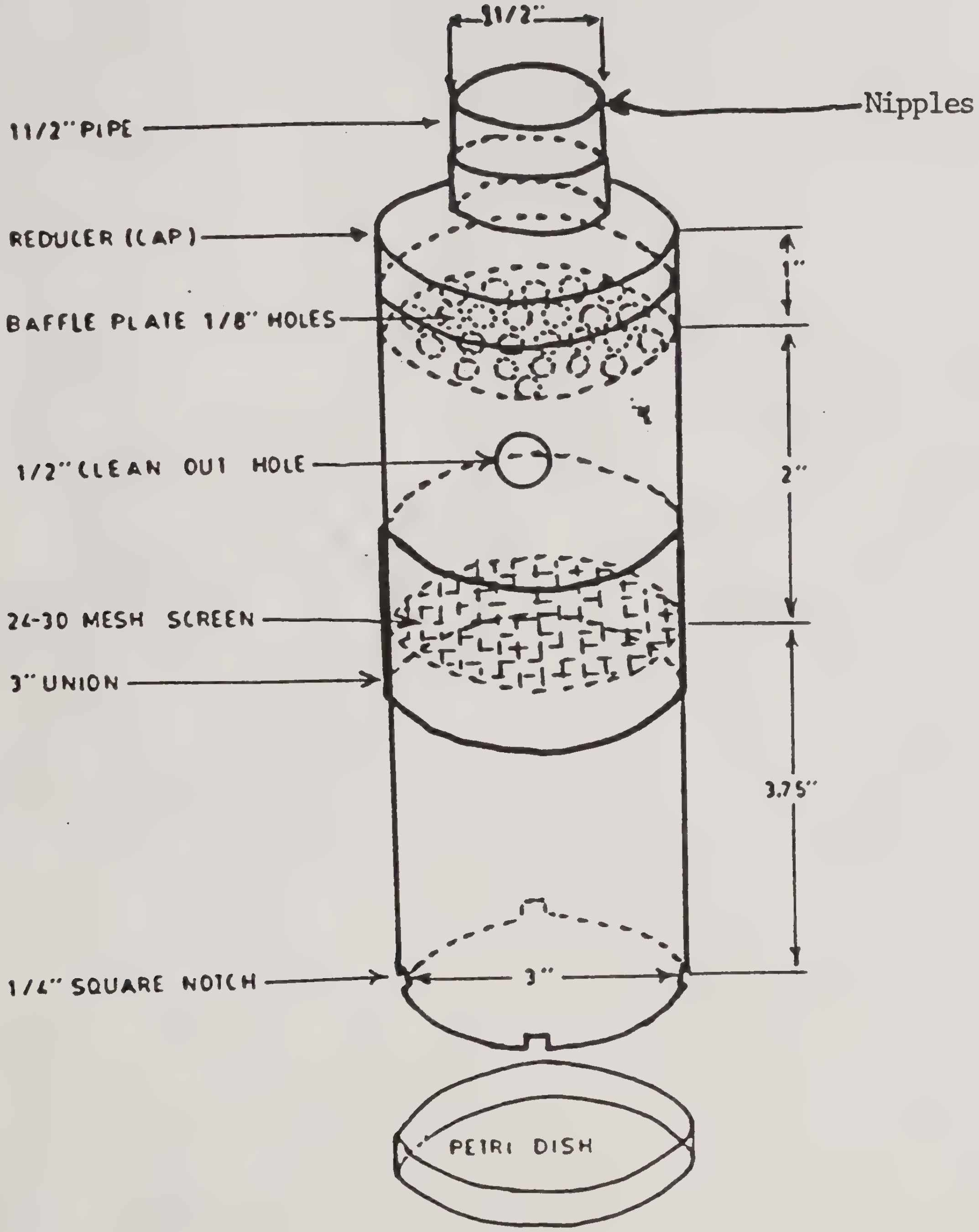


Figure 1--Dehairing chamber

2. A swivel joint can be made and attached to the 1 1/2 inch pipe by soldering two trees together at right angles, making a swivel for the copper to swing on. This is an optional attachment but is recommended for ease of operation. (Figure 2)

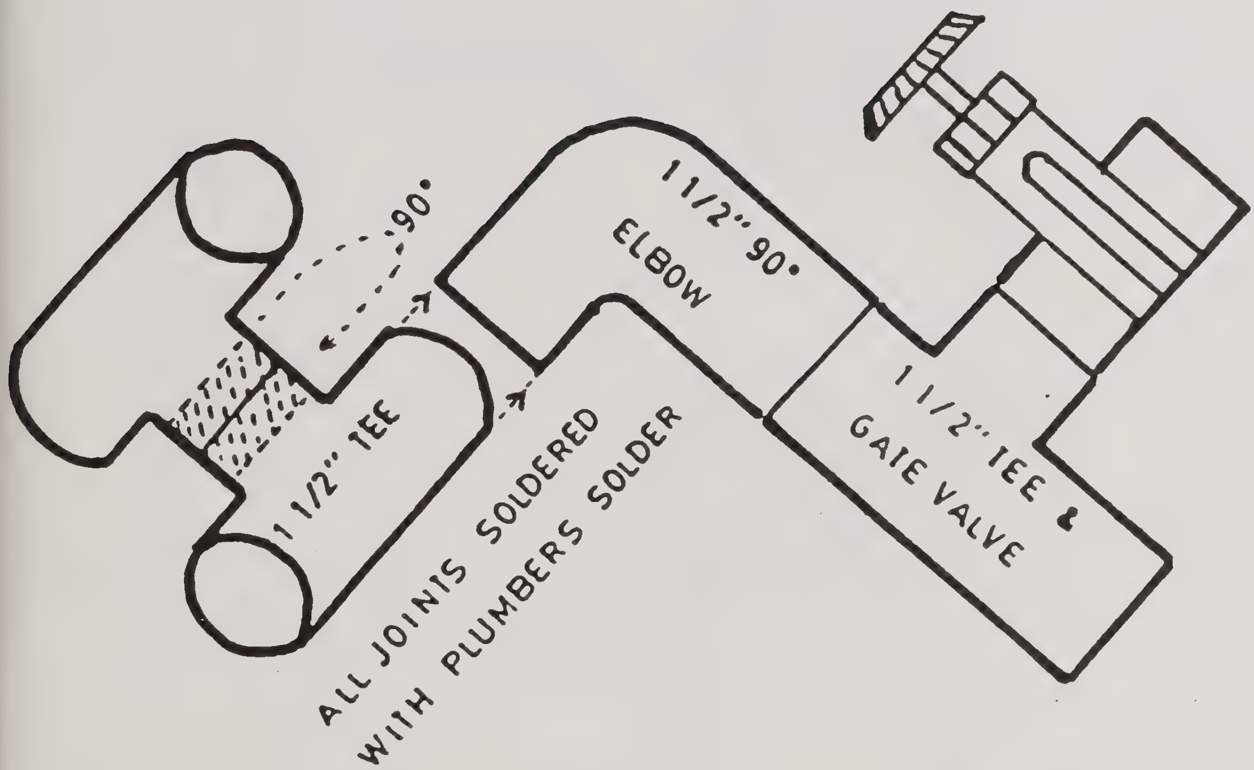


Figure 2--Example of gate valve and swivel joint made from copper trees. All joints are soldered with plumber's solder.

3. The swivel joint gate valve and dehairing chamber are fastened together to make one unit, dehairing assemblage. (Figure 3)

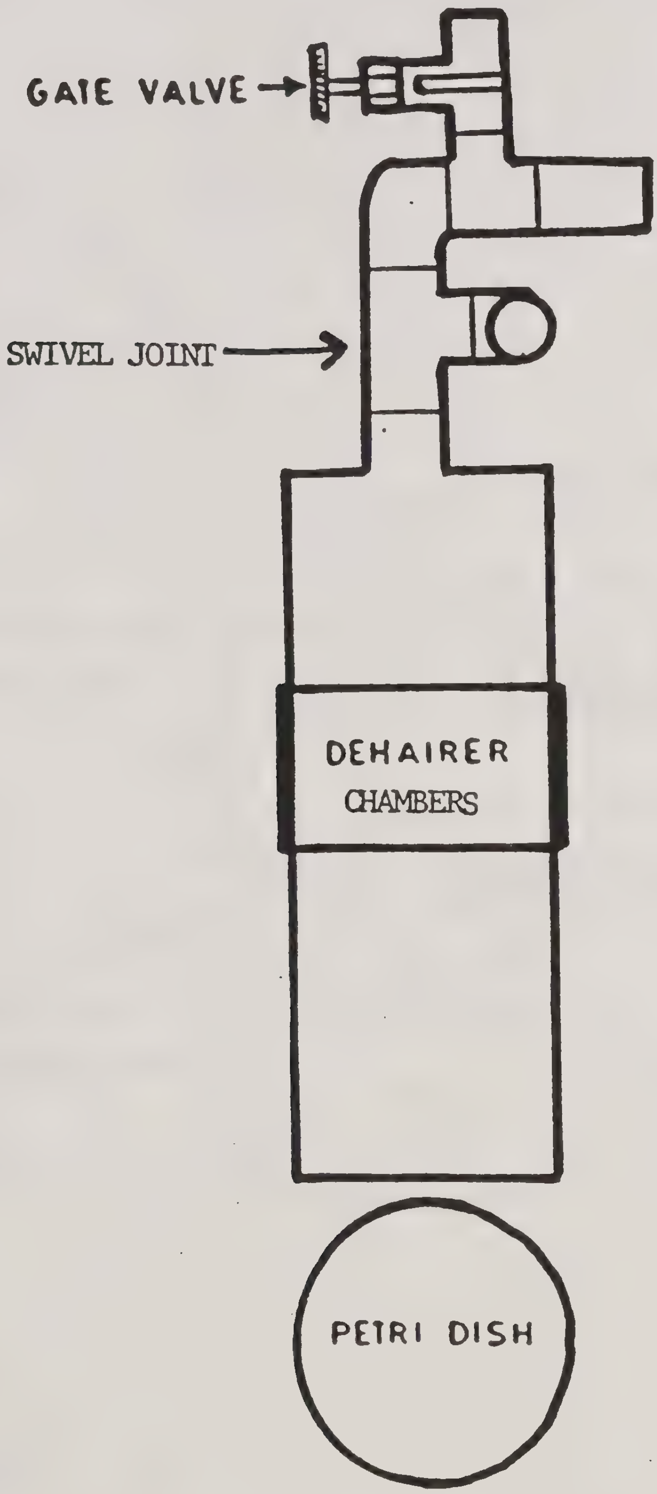


Figure 3--Egg mass dehairer assembled for use on rack.

4. The dehairing assemblage is mounted vertically approximately 9-10" above a table top so that a collection pot can be placed under it. (Figure 4)

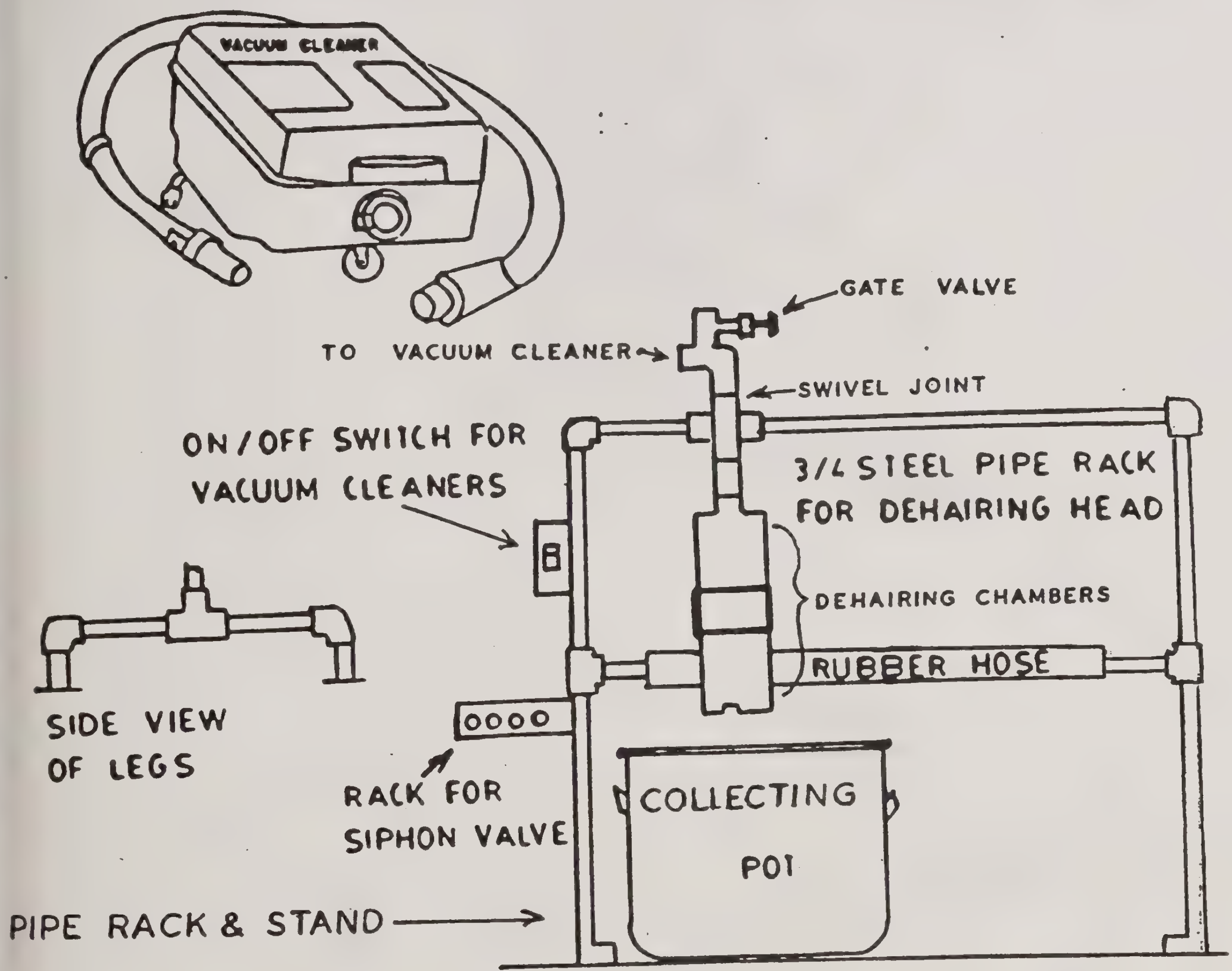


Figure 4--Egg dehairing machine mounted on a pipe rack.

The egg counter consists of a pipette, siphon valve, plastic tube, and vacuum pump. (Figure 5) The pump, a pressure vacuum type made by Gast Manufacturing Company, is fitted with four feet of 1/4 inch rubber vacuum tubing. The siphon valve (S.P. TRU Flate, made by Parker Automotive Industries, is attached to the free end of the tube for easy control of vacuum suction. (Figure 5b) The pipette is fitted at the base with a 1/2 inch length of rubber tubing to insure an air-tight joint with the siphon valve. A piece of 24 gauge copper screening is coiled and placed in the pipette at the zero mark to prevent loss of eggs into the vacuum pump. (Figure 5)

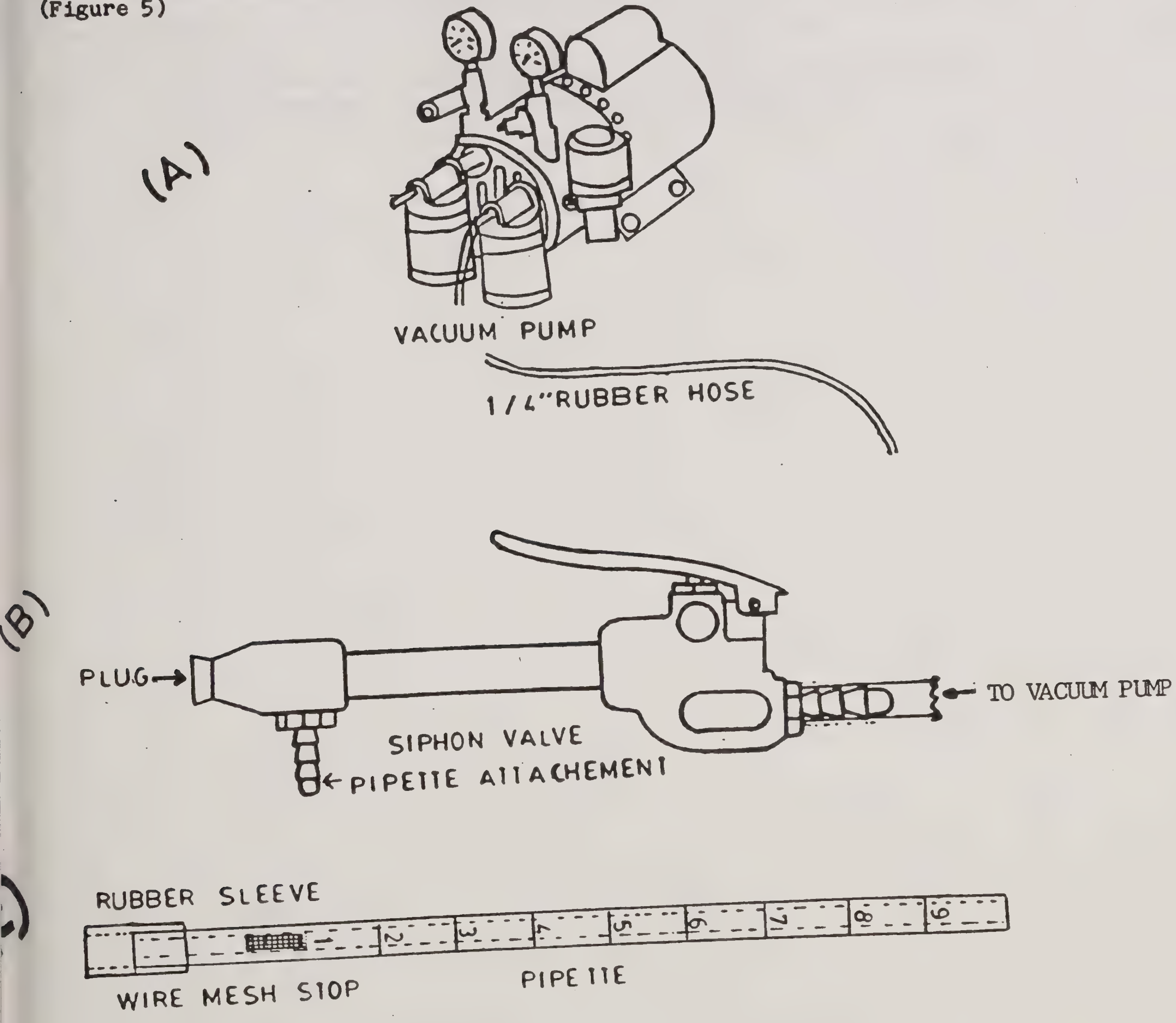


Figure 5--Gypsy moth egg counter (A) vacuum pump; (B) siphon valve; (C) pipette with plastic collar and wire mesh egg stop.

UNITED STATES
DEPARTMENT OF
AGRICULTURE

Forest Service

Northeastern Area

Predicting Defoliation by Estimating Gypsy Moth Egg Mass Viability

DRAFT MANUSCRIPT

March 1988

Prepared by: George Saufley, Forestry Technician, USDA Forest Service, Forest Pest Management, P.O. Box 640, Durham, New Hampshire

INTRODUCTION

In 1971 a method was developed by the author for cleaning, counting and sorting the eggs in gypsy moth egg masses. During sorting, the proportion of eggs expected to hatch is determined. The purpose of this paper is to update the original description of the method and to explain how to use the resulting data to ~~assess~~ Assess defoliation.

OBJECTIVES

1. To separate and sort eggs so that the health of the egg mass can be examined and egg mass condition evaluated as to its potential to hatch.

2. To distinguish eggs that will hatch from parasitized sterile or diseased eggs.

3. To use these estimates to assess egg mass health within geographic boundaries.

4. To incorporate these estimates into biological evaluations and control strategies.

METHODS AND MATERIALS

1. The "dehairing" and sorting apparatus consisted of a vacuum cleaner and a home-made attachment shown in Figures 1, 2 and 3. The dehairing machinery was redesigned from Pete Minor's dehairing device used in New Jersey. The attachment consisted of copper pipe, made up in the following manner.

a) An 8 inch copper pipe, (3") diameter is cut into 2 pieces, each one 4" inches long. At one end of the 3 inch copper pipe a 3" to 1 1/2" copper or brass reducer is attached to the 4" x 3" pipe. Inside the pipe, one inch from the reducer end, is attached a baffle plate with 130-140 evenly distributed 1/8" holes. At the other end of the 3" pipe, a 24-30 mesh screen is attached and the bottom 4" section of pipe is fastened to the top half via a union. All joints can be soldered with plumber's solder. Half way up the 4" pipe section a 1/2" hole is drilled to form a clean out port. The hole is taped shut when processing eggs. Four 1/4" evenly distributed square notches are cut in the bottom (end of 4" pipe). A 1 1/2" x 1 1/2" nipple is soldered to the 3 1/2" x 1 1/2" reducer for attaching to vacuum cleaner and rack. A petri dish is placed on the notched end of the dehairing head when the equipment is used (see Figure 1).

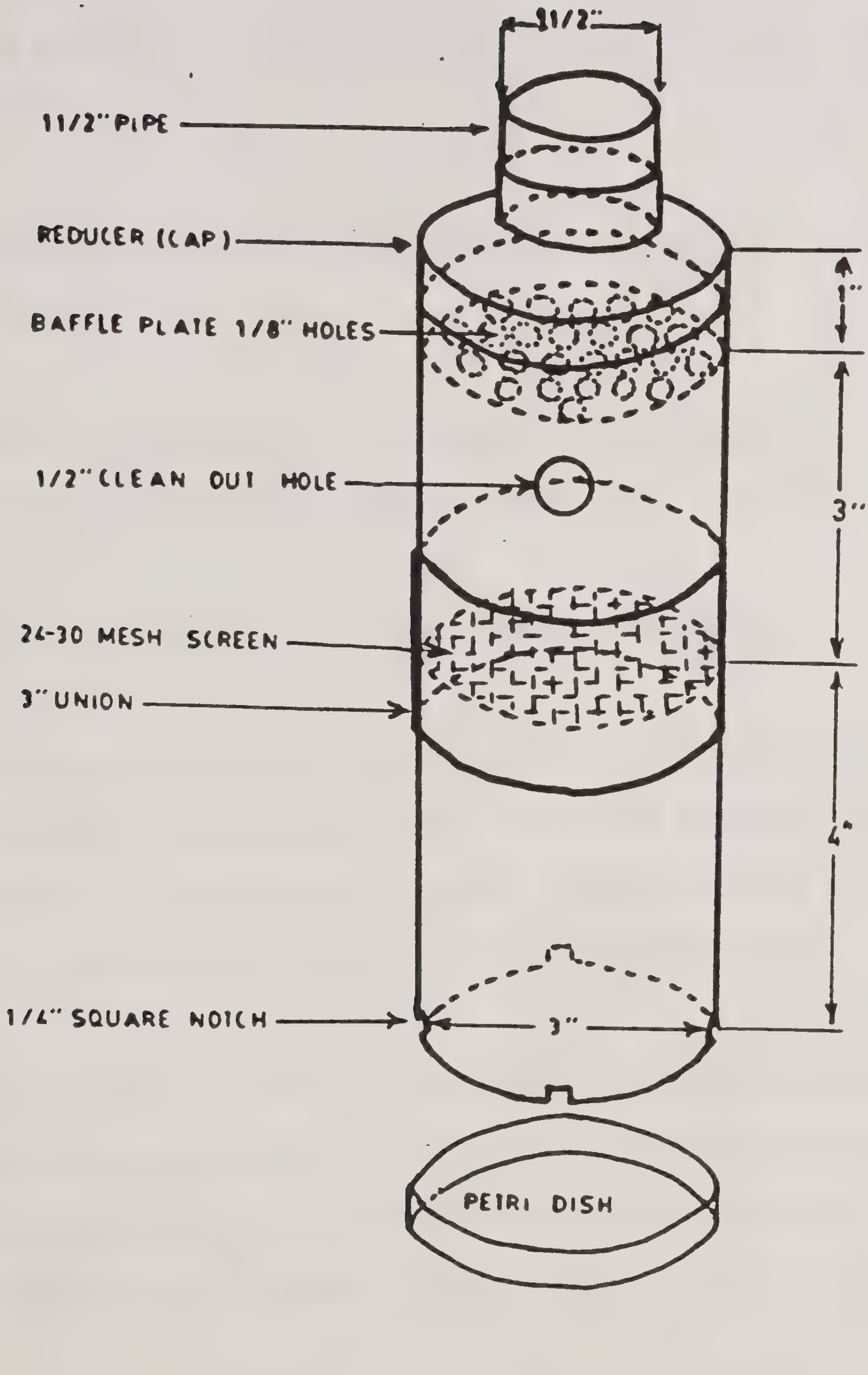


Figure #1—Dehairing head

2. To allow the dehairing unit to be tipped up, a hinge can be made from 1 1/2" pipe by soldering 2 tees and a nipple together at right angles (see Figure 2).

3. The hinge, gate, valve, and "dehairer" are fastened together to make one unit (see Figure 3).

4. The egg dehairer is mounted vertically approximately 9-10" above a table top so that a cooking pot can be placed under it (see Figure 4).

1. The dehairing head is operated in the following manner:

   a) Turn vacuum cleaner on and place petri dish containing egg cluster on the open end of the dehairing head. The vacuum holds the dish in place while the eggs are being cleaned. Place a cooking pot under the dehairer to prevent egg loss in case the equipment stops.

   b) The plastic lid is rotated 3-4 times while the eggs are bounced about in the copper can and the hairs are sucked through the screen into the vacuum cleaner. The can is tipped up horizontally to see if the hairs are sucked through the screen into the vacuum cleaner.

   c) When the hairs are cleaned away the gate valve is closed to the point where the whole eggs drop into the 4 quart cooking pot. The copper can is tapped against the rack to collect all the whole eggs.

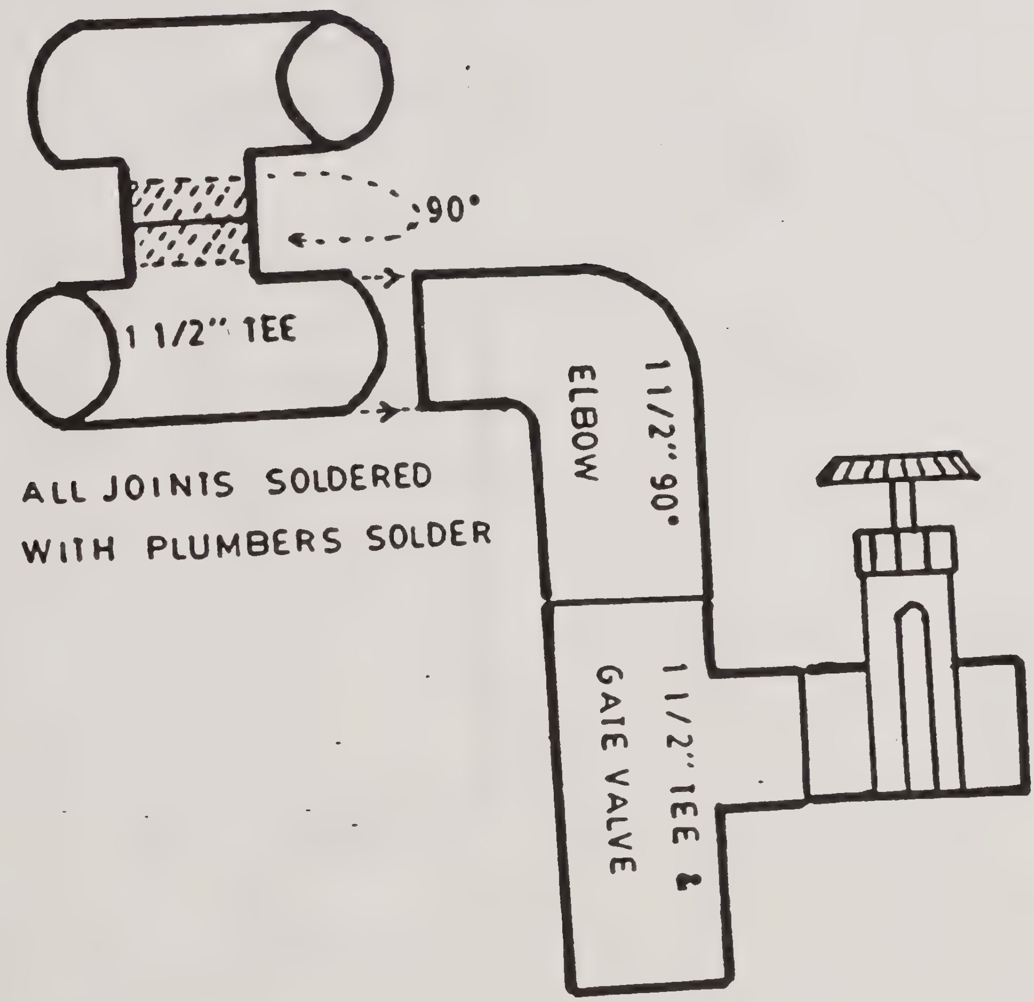


Figure #2--Example of gate valve and hinge made from copper tees

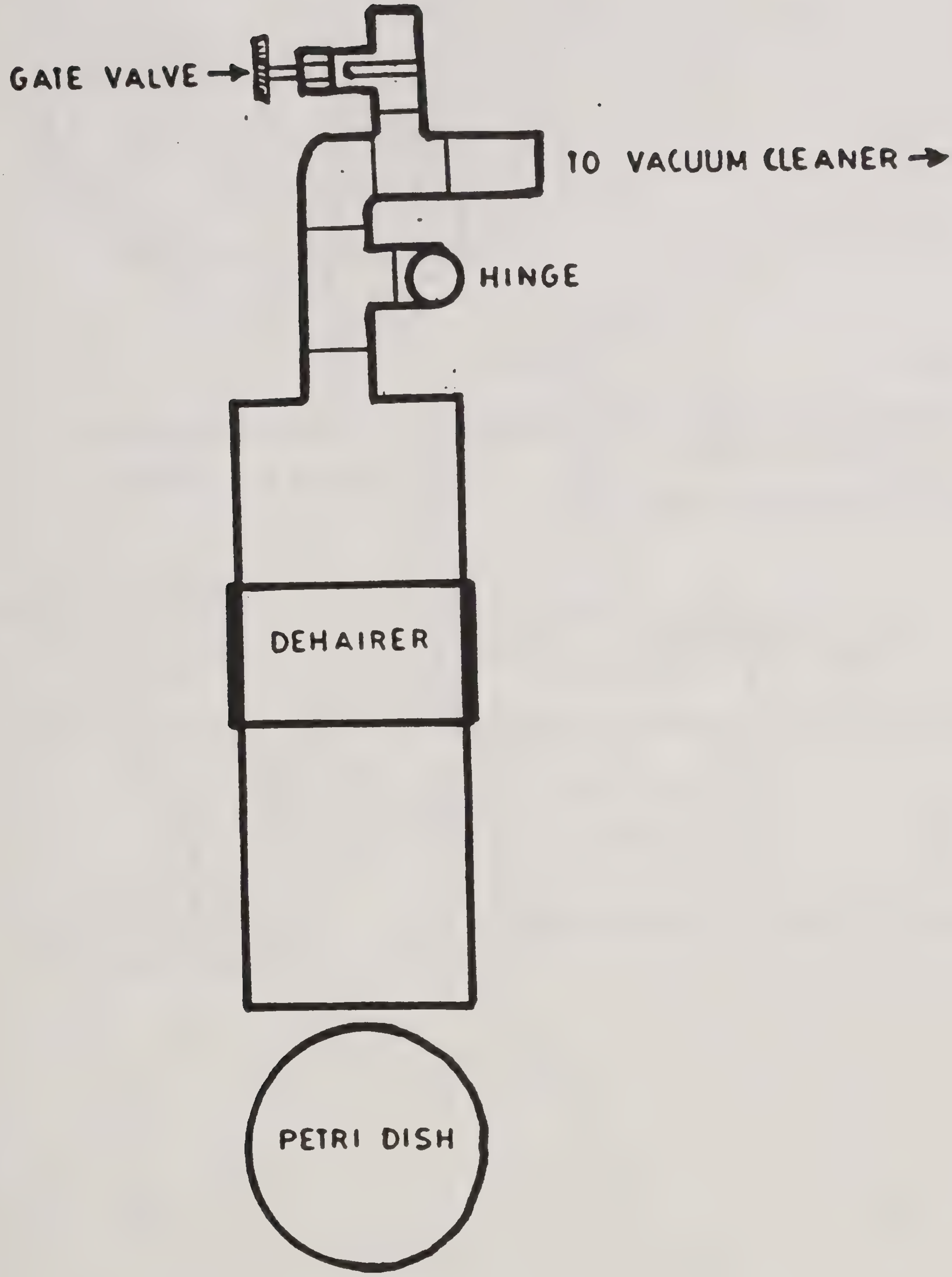


Figure #3--Egg mass dehairer and plumbing assembled for use on rack.

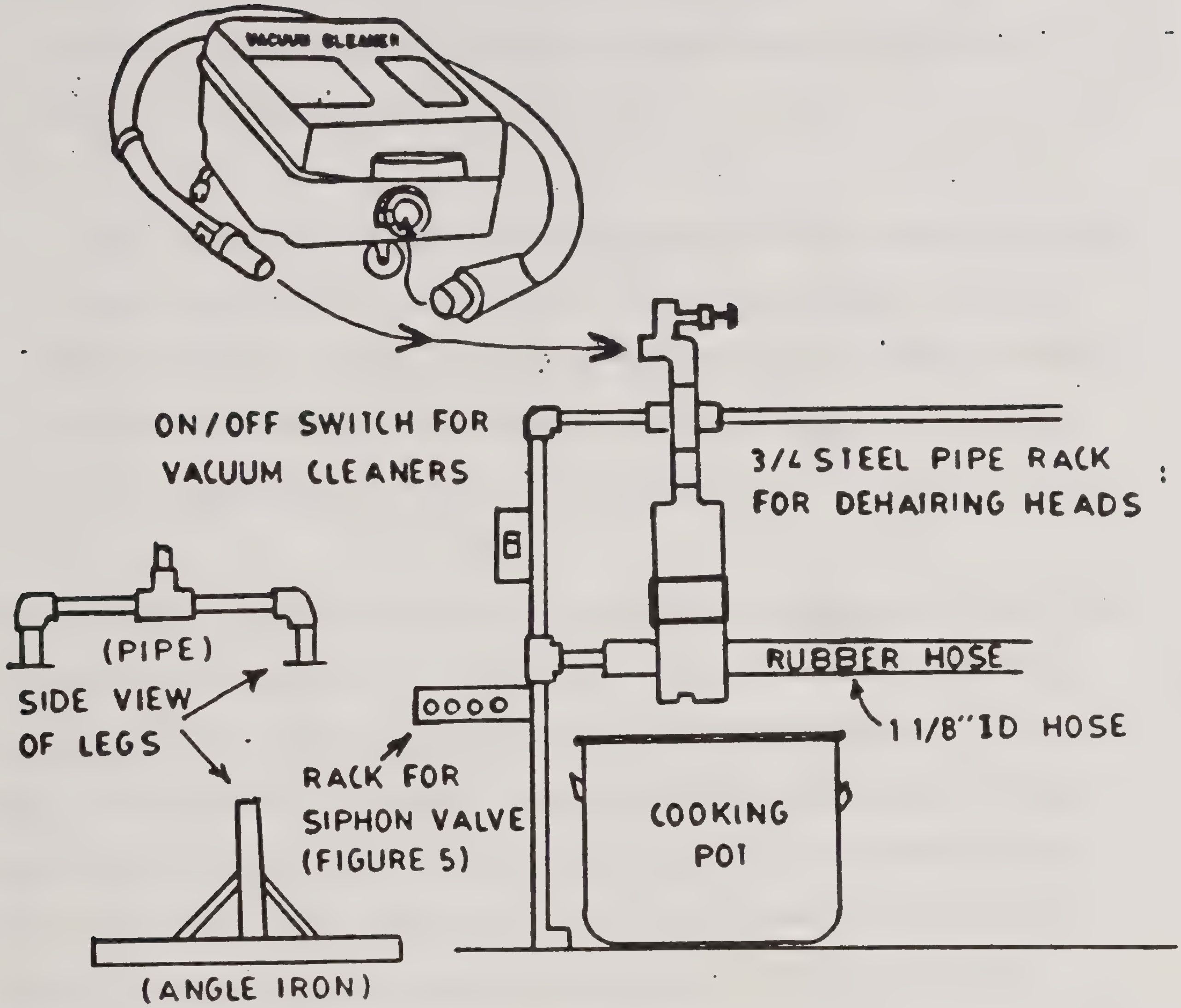


Figure #4—Egg dehairing machine mounted on a pipe rack.

d) A second 4 quart cooking pot is placed under the "dehairer" and the vacuum is turned off. The dehairer is tapped against the rack to collect the hollow eggs.

It requires about 30 seconds to a minute and a half to dehair and sort the eggs into whole and hollow eggs. The counting device described next separates the eggs from some of the bark left on the egg masses. At least 70% humidity is recommended to keep the eggs from bouncing around or failing to drop into the cooking pot.

2. The egg counter consists of a pipette, a siphon valve, a plastic tube, and a vacuum pump (see Figure 5). The pump, a pressure vacuum type made by Gast Manufacturing company, is fitted with a four foot length of 1/4 inch rubber vacuum tubing. The siphon valve (S.P. TRU Flate), made by Parker Automotive Industries, is attached to the free end of the tube for easy control of vacuum. The pipette is fitted at the base with a 1/2 inch length of rubber tubing to insure an air-tight joint with the siphon valve. A piece of 24 gauge copper screening is coiled and placed in the pipette at the zero mark to prevent loss of eggs into the vacuum pump (see Figure 6).

Egg counting immediately follows dehairing and sorting. The pipette, with vacuum pump on, is moved about in the cooking pot to pick up all eggs while avoiding bark flakes.

When all eggs in a cooking pot were picked up by the pipette, the volume was read and the number of eggs determined from the appropriate chart (Appendix A & B). It is easier to tap the pipette against a 250^{ml} beaker causing glass to "ring" and release the eggs. If a computer or programmable calculator is used, only the measurements and scope estimates are entered into the machine and the computer prints out the results while another egg mass is being processed or scoped. The computer and printer need not be expensive because the program is short.

3. Separation of parasitized, hatched and aborted eggs. When sorting the eggs into (2) major groups, whole and hollow, we found that a few eggs may be sorted into either the whole or hollow category. The vacuum gate valve can be adjusted so that the ambiguous eggs can be put into either the whole or hollow category. In 1972, a Chi square was applied to test for a significant loss of whole eggs into the other category at the 95% level. If one wants an estimate for parasitized eggs containing <u>Ooencyctus kuwanai</u>, mummified caterpillars, bacteria or fungus infected, or dried up eggs, then you can examine the hollow eggs under a binocular microscope for percentages in each category.

   To determine how many eggs can potentially hatch, examine the whole egg category. These whole eggs are examined under a binocular dissecting microscope usually at 6-12 power with an 8-10 X eye piece to get a wider field and examine more eggs. A 15 watt light bulb focused under the eggs will show a shadow of a caterpillar embryo. A group of 30-50 eggs is examined under a low power scope to get a ratio of eggs that have

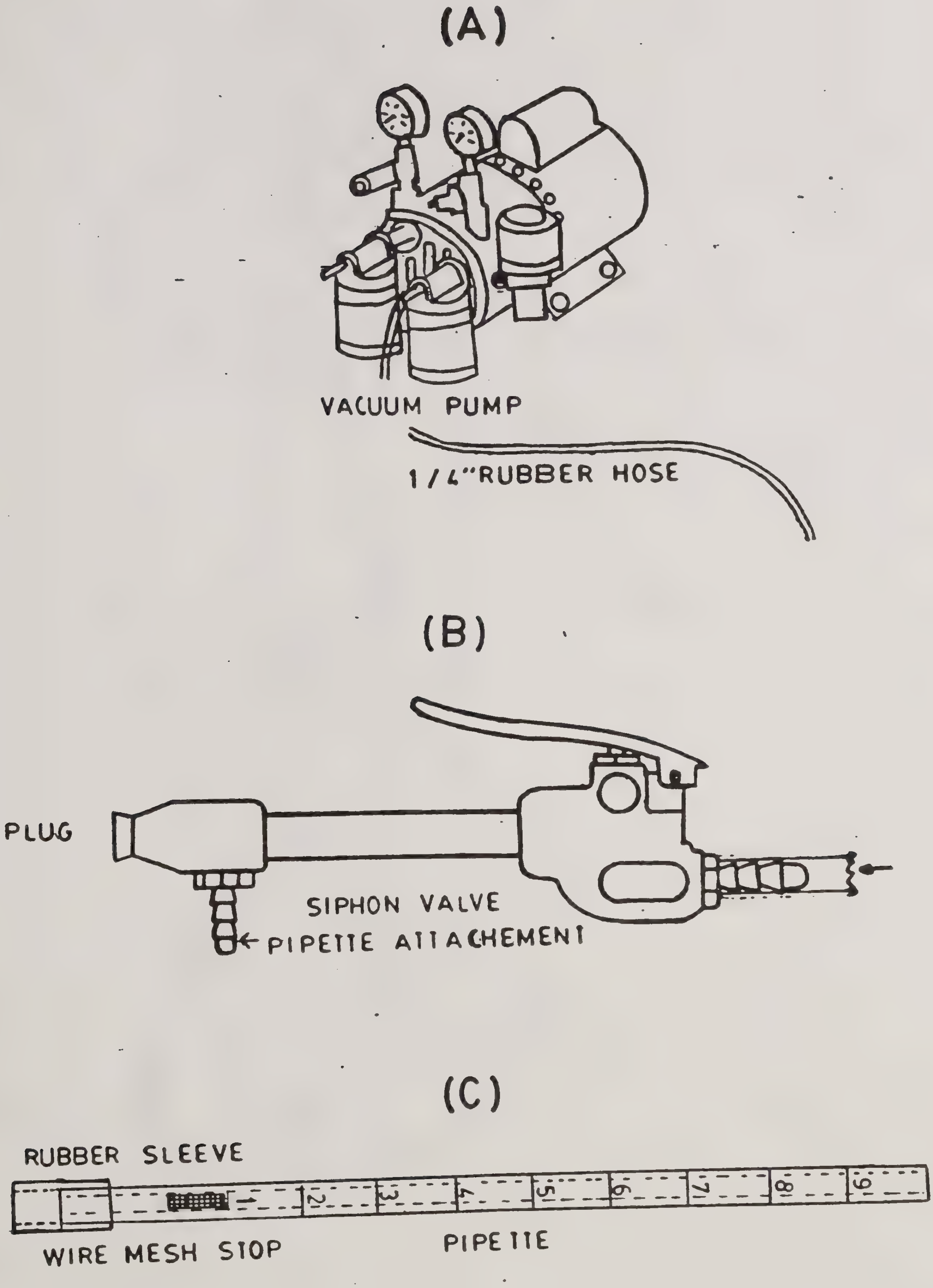


Figure 5 Gypsy moth egg counter (A) vacuum pump (B) siphon valve (C) pipette with plastic collar and wire mesh egg stop.

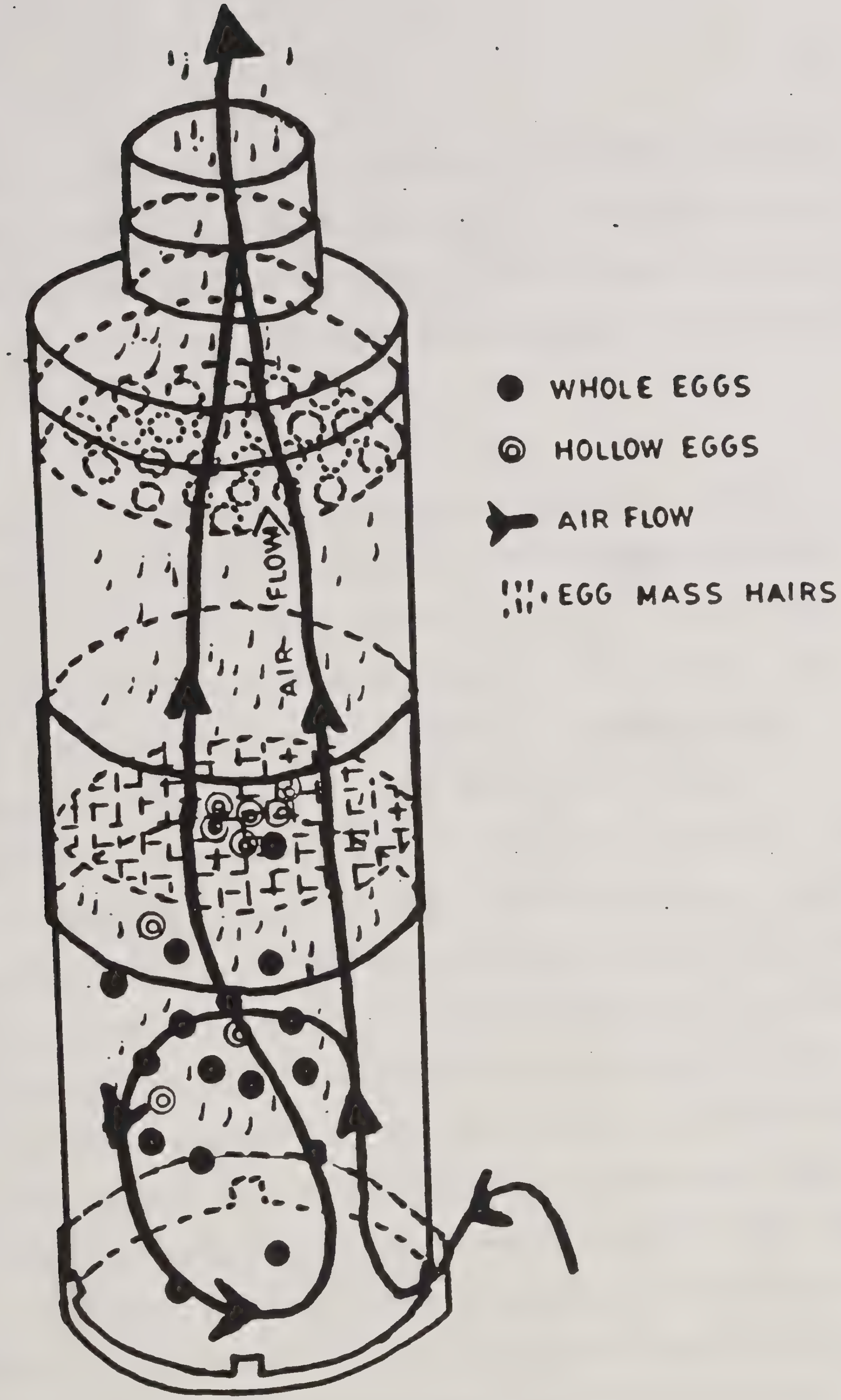
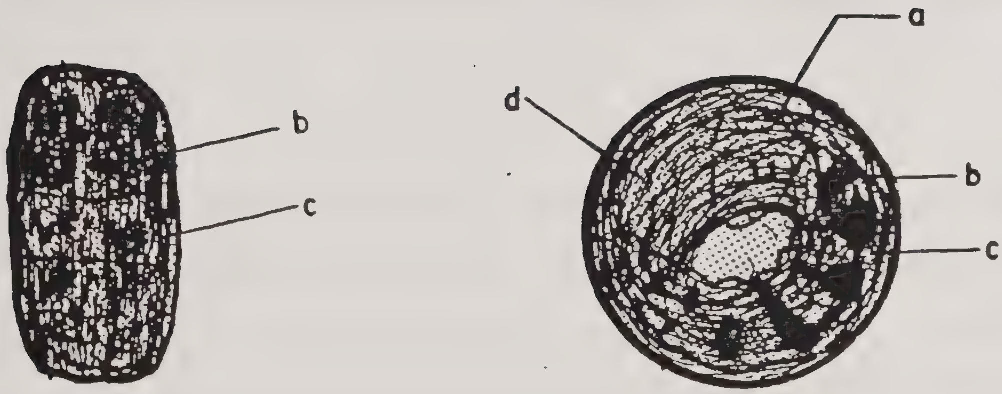


Figure 6 - Diagram of dehairing process

caterpillar embryos and eggs that have no potential for hatching(such as larval parasites, unfertilized and diseased eggs.) This ratio is used to determine the potential hatch of the egg mass. Use as many fields as necessary to get a reasonable estimate of the condition of the egg mass (see Figure 7).

The eggs are candled for abnormalities in the same manner farmers used to candle poultry eggs. A silhouette of an embryo in the egg makes a statement about the egg's health. However, it is difficult to see into an egg without dissecting it. One solution is to place known healthy eggs one layer thick on a slide and with a dropper place some diluted black ink between the eggs. This technique allows the examiner to see the egg's contents and eventually get used to looking at egg clusters without ink. If the egg mass cannot be easily separated into (2) distinct categories of eggs, then suspect the egg cluster's health. Both whole and hollow eggs may need examining to determine the cause. These egg masses are usually not healthy and have high levels of parasitism, winter kill or disease, but expect a small percentage of the eggs to hatch. If the population has been high, parasitism is high, and the egg masses are small, along with the phenomenon of non-discrete separation of healthy and unhealthy eggs. Then a population collapse is likely because of a depleted food supply. If the division between the whole and hollow is pronounced and the hollow eggs represent 1-10% of egg masses that are large and uniformly scattered throughout a preferred host stand; and is the whole category within the egg cluster indicates that 80% or more the egg cluster has the potential to hatch, then the population is likely to build. This condition may be supported by increased pheromone trap catch in the area.



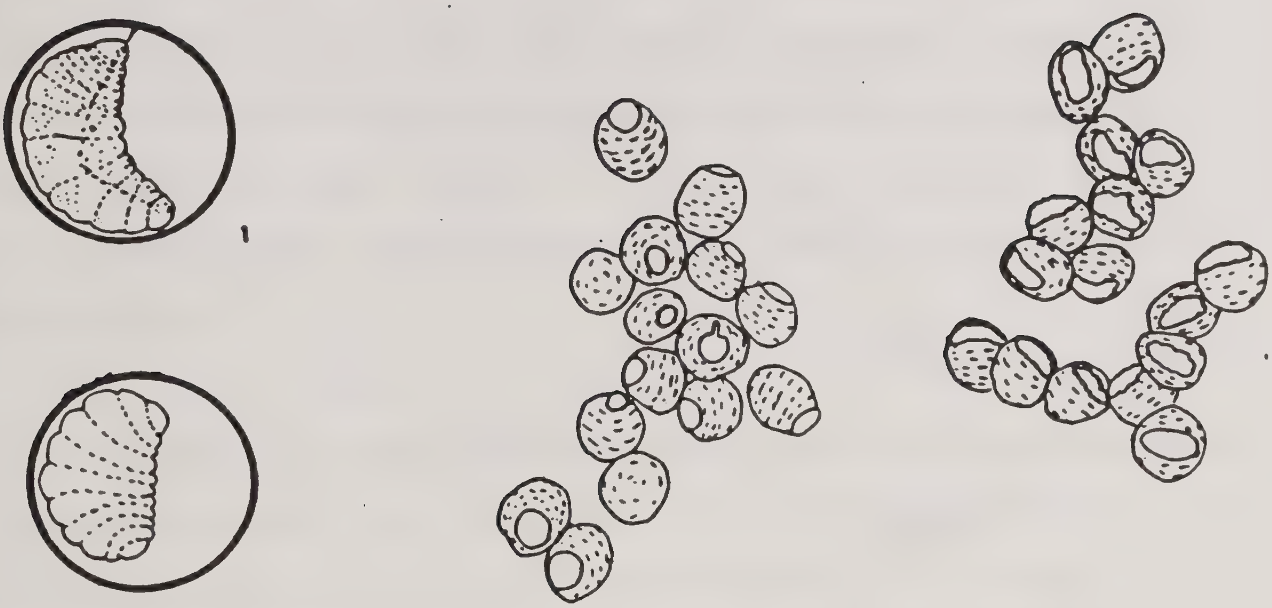
a) Shadow head capsule
b) Internal organs, structure inside egg
c) Thorax and abdomen
d) Larval hairs

Figure 7 - Examples of gypsy moth embryos in eggs

Processed and unprocessed egg masses were reared in an environmental chamber to get an estimate of over wintering mortality and the results vary from location to location and within populations. Comparisons of processed and unprocessed egg masses classed according to size were made to see if the occular method was a reliable indication of egg health. Eggs used in the comparison were hatched in an environmental chamber. When observations were averaged, there was no difference between those that we expected to hatch and those eggs that actually hatched.

The remainder of whole eggs were placed in petri dishes, labeled, and placed in a styrofoam box along with damp paper toweling sprayed with 1% sodium benzoate solution and stored in a walk-in cooler for 3 months around 34^0. Next the eggs were removed and reared in an environmental chamber at 80^0 F 70% RH at 14, 10 photo period. If the average temperature and RH approximate early summer weather the eggs will hatch. The eggs remaining from the inspection process are a better indicator of hatch, because prolonged exposure to intense light will damage the eggs.

Both whole and hollow eggs may be examined under a 150 power compound binocular microscope for hatch potential. Hatched and emerged parasitized eggs as well as completely dry eggs are separated out with the hollow eggs. Eggs with parasites that have not emerged or eggs containing most of their original weight that are diseased may drop as whole eggs. These are examined when the whole eggs are scoped. The dark brown caterpillars in the eggs are healthy gypsy moth larvae. If a lot of white or light brown larvae show up in the egg cluster, they should be reared for parasites (see Figure 8).



A

Parasite Embryos
Lighter in color/white

B

O. Kuwanai Emergence Hole

C

Gypsy Moth
Emergence Hole

(in most cases parasite is difficult to see in egg because of hairs, egg structures

a1 - Ooancyrtus kuwanai
a2 - Anastatus bifasciatus

Figure 8 - a) Containing parasites
b) O. Kuwanai emergence hole
c) Gypsy moth emergence hole

SUMMARY

Field counting of egg clusters alone only provides estimates of egg clusters per given area with no indication as to the expected hatch of that cluster. Rearing egg masses will also indicate the expected hatch of that egg cluster. However, rearing egg masses through diapause takes time that could be used to prepare for control.

Dehairing, sorting and estimating the proportions of eggs in a gypsy moth mass that have the potential to hatch in a geographical area is an indication of the health of egg masses for that area at the time that the survey is being conducted. A person can process 45-90 + egg masses per day using the equipment and data analysis described.

The number or percent of eggs that will hatch in an egg mass can be incorporated into on-going biological evaluations to improve the chances of predicting the outcome of gypsy moth populations. A more accurate prediction scheme gives the land manager a wider choice of alternatives, and a more reliable prediction system provides a greater chance for a better benefit to cost ratio with gypsy moth control.

4. Calculating egg mass viability or health:

a) Dehair, sort and volumetrically count whole and hollow eggs. Whole + hollow may be scoped if necessary.

b) Total eggs per mass = whole + hollow eggs.

(1) From whole eggs examine 50 or so eggs that comfortably fit into microscope field. Examine 1 or 2 fields.

c) # est viable eggs in scope fields = total eggs in scope fields minus eggs examined that will not hatch. (Formula can be other way around)

d) Ratio of eggs that will hatch = <u>est viable eggs in scope fields</u>/total eggs examined in scope fields.

e) # est eggs hatch per mass = total whole eggs times percent of eggs that will hatch.

f) % of eggs that will hatch per mass = <u># est eggs hatch per mass</u>/total eggs per mass x 100.

REFERENCES

Saufley, George C. 1972. Gypsy moth eggs, a method for cleaning, counting and sorting: Office Report, USA Forest Service, NA-S&PF. p. 21.

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